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THE DEVELOPMENT OF ISHNOCHITON

A THESIS

submitted to the Faculty of the Department of Philosophy,

in candidacy for the degree of

DOCTOR OF PHILOSOPHY,

(Department of Zoology),

May 9, 1898.

by

HAROLD HEATH.

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The following paper on the embryology of Ishnochi-
ton was undertaken primarily with the view of determin -
ing as far as possible the accurate origin and develop -
ment of the germ layers. As a result of such a study it
was hoped some light might be thrown on the phylogeny of
the mollusca, more especially with regard to the relation-
ship between this group and the annelids. The work has
expanded beyond my expectations, and owing to this fact I
have in the following pages paid particular attention to
the cell lineage and the external features of the devel-
opment through the formation of the trochophore and its
metamorphosis, up to the assumption of the adult form. The
development of internal organs only in so far as it bears
directly upon the complete understanding of external
structures has been considered. This latter phase of the
problem I hope to be able to present at another time, pay-
ing more particular attention to the development beyond
the oldest stages considered in this paper.

The work was begun in the summer of 1895 at the Hop-
kins Seaside Laboratory, Pacific Grove, California, and
during the following year, so far as my duties would per-

The material for this work was collected at the Hopkins Seaside Laboratory on Monterey Bay, California, about one hundred and twenty miles south of San Francisco. The north and east shores of the bay are sandy, but upon the south a granite formation breaks through the cretaceous conglomerate rises above the sea level from ten to fifty feet. The cliffs, together with the outlying islands, and the innumerable boulders at their base, support a prodigious number of plant and animal forms. Not less than twenty species¹ of chiton occur in this situation, and several of these in the greatest abundance. The eggs of one spe-

1. Professor Pilsbry, of the Academy of Sciences, Philadelphia, has kindly identified the following most important species collected in Monterey Bay:-

Callistochiton crassicosiatus.
" palmatus var. mirabilis, Pils.

Cryptochiton stelleri, Midd.

Cyanoplax Nuttallii, Cpr.

Ishnochiton Cooperi.

" Magdalensis, Hinds.

" Mertensii, Midd.

" radians.

regularis Cpr.

Katharina tunicata Wood.

Lepidopleurus rugatus.

Middendorfia Thomasii Pils.

Mopalia ciliata Sowb.

" lignosa Gld.

" muscosa.

Nuttallina californica.

Palochiton lanuginosus.

Placiphorella velata Cpr.

Tonicella lineata Wood.

cies, *Ishnochiton magdalenensis*, were collected, and their study forms the subject matter of the present paper.

Ishnochiton magdalenensis, more often called *Stenoradsia magdalenensis*, was first described by Hinds ('44) from specimens obtained from Magdalena Bay, Lower California. From this locality it extends to Monterey Bay, where it is probably the most abundant species of chiton, and in gradually diminishing numbers we find it for a distance of one hundred and fifty miles to the northward. Thus its range is from Magdalena Bay, Lower California, to Bolinas Bay, a few miles north of San Francisco, California, or a total distance of about twenty five hundred miles.

All chitons are probably vegetable feeders, and in Monterey Bay and vicinity the majority are certainly nocturnal, withdrawing into some shaded position upon the approach of day. *Katharina*, *Tonicella*, *Mullalina*, occupying exposed situations on the rocks, and concealing themselves but little, are apparently not highly sensitive to light; *Mopalia* and *Cryptochiton* will remain out on their feeding grounds only when the day is foggy or dark; the remainder of the chitons mentioned in the list conceal themselves

under loose stones or in crevices among the rocks, but none appear so highly sensitive as *magdalenensis*. During the day this species may be found in numbers of a score or more under such boulders as lie between tide marks, half buried in the sand, and overgrown with various seaweeds. During the night they come out to feed on this vegetation, sometimes emerging completely, but more often just far enough to eat those plants lying next to the sand. In these positions they may be found in the early morning, but by sunrise all traces of them have disappeared.

The proboscis of the adults appears highly sensitive to light. Until the *Ishnochiton* are about 4 c.m. in length it remains completely exposed as in other chitons, but about this time a black or dark green pigment is deposited in the anterior eighth of the foot, and in proportion as it deposits the foot is drawn more and more over the mouth, until finally the dark end of the foot completely conceals the mouth. I am unable to suggest a reasonable explanation of the above mentioned peculiarity.

Breeding Habits.

The eggs of *Ishnochiton* are laid on those days of May and June when the low tides come in the early morning. Unlike any chiton yet described, so far as I can learn, it does not lay its eggs singly, but in "jelly masses." The oviducts between the ovary and a point 8 or 10 m.m. from their exterior openings are modified to form albumen glands. Just before the breeding season these become greatly distended and the eggs passing out from the ovary are surrounded by the secreted albumen and become moulded by the lower end of the oviduct into strings each with a diameter of 5 - 4 m.m. and a length of about 77 c.m. Along one side of the string is a strip of albumen in which there are no eggs. It is not in the state of tension that exists in the remaining albumen packed with ova and consequently the strings as they are laid assume the form of spirals.

It is difficult to find *Ishnochiton* in the act of egg-laying, and the observations I have made along this line are few in number. They have never bred in captivity, and the process in the normal state is completed us-

ually before daylight. Upon one occasion only have I seen the method. It was in the early dawn and several specimens were found out of water, having been left by the retreating tide,--a fact which probably accounts for their tardiness, since all the submerged ones had laid. In each case the females were attached to rocks, with the posterior half of the body protruded above the sand. The free edges of the foot were expanded in such a way that they sent wing-like processes across the gill furrows to the mantle. The gills thus occupied tubular cavities, the ends of which were open owing to a slight elevation of the posterior border of the mantle. The egg strings had issued through the tubes thus formed, and several specimens that continued laying in the laboratory showed the rate to be 7 inches per hour, occupying, if this rate be normal and uniform, nearly five hours in laying egg strings 31 inches long (see diagram).

In many cases the strings are found in the greatest profusion attached to the seaweeds (fucus, corallines etc) that cover the rocks in this region. It may be that they were placed there directly, or were washed into this po-

sition. I cannot say which method obtains, but I am inclined to the latter, since the chiton when laying in the aquarium are perfectly quiet. The egg strings are not fastened, simply caught on the leaves and stems and the heavy waves wash them away and break them into fragments so that it is scarcely possible on the next day to find pieces longer than one or two inches, and after a week has elapsed almost all traces have disappeared.

The chiton, along a considerable extent of coast, lay on the same day. For example, on the morning of June 9 eggs were found along a quarter of a mile of coast; on June 13 over a somewhat larger area; and on the 23rd. for more than a mile. In each case a large number of specimens were examined and all were found to have shed their sex products. In tide pools, as I once observed, females of *Mopalia lignosa*, though distant several feet from the males, will lay almost as soon as the sperm diffuses to them; the same is true of a species of capitibranch worm (probably *Cirratulus*.) of the west coast. It may be that in the case of *Ishnochiton* one of the reasons the eggs are laid in such definite localities is owing to the dif-

fusion of sperm by the tidal currents, though the fact that all are subjected to similar conditions, e.g. quietness of water, time of month or year, temperature, must be of high importance.

By compressing portions of the egg string one half inch in length under a ruled cover glass, and counting the eggs, a very close estimate of the total number of eggs laid by each individual may be made. This was done in several cases, and gave 621,955, and 1561 as the lowest, average and highest number. Considering that every chiton lays two strings each with an average length of 31 inches, the total number of eggs laid in the above examples is 101804, 115940, and 193564.

Despite the fact that such a vast number of eggs are laid, only a very few develop into adult chiton. A careful search six months after the egg laying season will reveal but a relatively small number of the young, which by this time have reached a total length of about 8 m.m. What has been said for *Ishnochiton* in this connection, bears with yet greater force in the case of *Katharina*. In some places in the vicinity of Monterey Bay the rocks are

literally covered with the adults, and the number of eggs laid by every female must be fully twice ¹ the average number of eggs laid by Ishnochiton, yet the following winter or spring shows a surprisingly small number of young specimens.

The development proceeds with considerable rapidity. Twenty-four hours after the eggs are laid the larva commences to rotate within the egg membrane; six days later they break through the chorion and swim about freely for a period lasting from fifteen minutes to two hours. After this time they settle on the rocks and seaweeds, and undergoing a slight metamorphosis during the next three or four days they assume the external characters of the adult.

Methods.

Several methods of killing and fixing have been tried, by which the albumen in which the eggs are imbedded would not shrink when brought into alcohol. With the usual methods this shrinkage occurs, and the eggs become so contorted that the material is almost useless. Flem-
 1. I have made an approximate estimate of the number of ripe eggs in the ovary of Katharina, and I believe the above assertion is not far from correct.

ming gave excellent results. Specimens were killed and fixed for about six hours, and were then washed in water for about two hours more. By this time the albumen ceases to be viscid, and assumes a consistency like that of coagulated white of egg. The chorion has delicate forked processes projecting from it (Fig.1) to which the albumen strongly adheres, and upon stretching this latter with needles, the projections remaining fixed, the chorion splits at right angles to the tension and the egg drops out. By simply splitting the string longitudinally with a fine needle hundreds of eggs per minute may be freed from their membranes. They are then washed for another hour in water, run through the different grades of alcohol, and permanently preserved in 90% alcohol. Eggs killed in this way afford in direct sunlight wonderfully fine surface views. They are perfectly opaque, black as ebony: resting nuclei are fairly clear and mitotic figures are often indicated, and owing to the full round outlines of the cells their boundaries come out with the greatest distinctness, rendering the relations of the cells in general of comparatively easy interpretation. Under certain

circumstances the cell boundaries appear clearer when the egg is some shade of brown. In such cases treatment with hydrogen peroxide brings about the desired result.

For study the eggs are brought into a watch glass, and are prevented from rolling by placing in with them some finely cut camel's hair. In this way the eggs remain in position, and free hand drawings are readily made

Picro-sulphuric and picro-acetic also gave excellent results, especially the latter. but eggs must be kept in these fluids for five or six hours, otherwise in teasing out the eggs are so broken or distorted that their study is unprofitable; but after a period of six hours the albumen assumes a condition somewhat similar to that produced by Flemming's fluid, and the eggs may then be rid of their membranes. This latter process is rather slow after these fluids, but it may be done at any time within two weeks if the eggs are kept in a mixture of equal parts of 95% alcohol and 4% formalin.

For surface views Delafield's Haematoxylin with a light secondary stain of eosin gave the best results. Sections were made in paraffine and stained in Delafield or Biondi-Erlich. In running whole embryos through a clear-

ing agent, care must be exercised not to put them at once into one of high diffusibility, e.g. xylol, which reagent splits the cells asunder. Cedar oil answered best in all cases. In surface views the nuclei stained with Delafields Haematoxylin are quite distinct, but the eggs are filled with a finely granular yolk that often gives an indistinctness to the cell boundaries in later stages, and it is a tedious process to determine them, but by combining the study of eggs killed in Flemming's fluid, where the boundaries are clear, with a study of stained preparation a perfect interpretation is possible.

General sketch of development.

The cleavage of *Ishnochiton* is total, nearly equal, and the early cleavages conform to the radial type. The relation of the first two cleavage planes to the axes of the future embryo could not be determined since definite landmarks are lacking until the twenty-eight cell stage. Three quartettes of ectomeres are cut off from the macromeres and these constitute the entire ectoblast. The mesoblast forms the left posterior macromere at its fourth division, and the remaining products of this quar-

tette and the macromeres constitute the endoderm.

Up to the formation of the mesoblast in the seventy second cell stage a perfect radial symmetry exists. At this time however certain divisions in the posterior second quartette (first somatoblast) arise which to some extent destroy it, but in the upper hemisphere, on the other hand, radial symmetry persists at least until 120 cells are formed. Bilateral symmetry appears therefore at a rather late stage in the development, and after its appearance the transition from the radial to the bilateral condition is slow: in fact traces of the former persist until the close of the free swimming period.

Gastrulation occurs almost entirely by invagination which proceeds most rapidly on the anterior side of the blastophore where the cells are smaller. In the rapid movements of this process the mesoblast becomes pushed backward, almost posterior to the archenteric wall, and with the shifting, invagination on the posterior side practically ceases, while it continues on the anterior side with unabated vigor. Rapid cell divisions now arise on the posterior side of the embryo in the ectodermal

cells whereby the distance between the posterior borders of the blastopore and prototroch becomes constantly increased. In proportion as this increase takes place a corresponding decrease of the surface between the blastopore and prototroch occurs by means of the invagination process. Finally the ectodermal tract between the blastopore and velum enters into the formation of the stomodaeum, and the mouth consequently comes to lie immediately behind the prototroch.

During the first stages of the shifting of the embryonic axes, the prototroch, consisting of thirty four cells arranged in a double row along the embryo, become ciliated. As in the annelids, a gap at first occurs on the dorsal side, but this is filled by two cells from the anterior hemisphere. This organ continues functional until after the free swimming stage is passed, when it becomes pushed out of its position and is cast away.

By a slight invagination a few cells in the centre of the anterior hemisphere sink in a short distance and become attached to the cerebral ganglia, which arise as two ectoblastic thickenings in the head vesicle (Fig.)

From the bottom of this depression two compound flagellae arise, the whole structure forming the apical sense organ.

On the ventral side just posterior to the mouth the foot arises as a median undivided protruberance. It probably is formed to a slight extent by the second and third quartettes of the anterior side of the embryo, and to a greater degree by these same quartettes of the posterior side of the embryo, which have grown round to form the ventral side. On its anterior edge arises the opening of the "foot gland" (Kowalevski), an organ probably similar, in function at least, to its namesake in other molluscs.

About the time of the formation of the foot the first indication of a shell appears on the dorsal surface, consisting of rows of cells, which are destined to secrete the calcareous salts alternating with large cells apparently containing some mucous-like secretion. A cuticle forms above this region in which the valves of the shell and the spicules of the mantle are deposited. This shell is a cuticular structure constituting the tegmentum, the articulamentum arising much later. At first it occupies a position posterior to the prototroch but gradually it

extends onto the head vesicle and finally its anterior borders occupy a position but little posterior to the apical sense organ.

The larva, which escapes from the membranes before the calcareous portion of the shell commences to appear, has much the appearance of the adult, the hemispherical form of the head vesicle constituting the most apparent difference. This gradually flattens however, and ultimately forms the proboscis and the anterior part of the mantle furrow in the manner indicated by the figures, and this change completes the essential features of the metamorphosis.

The parts the various quartettes play in the developmental processes are given in the table on page .

Nomenclature

I have used Conklin's system of nomenclature throughout, basing it upon the same characters, and I cannot do better than quote his paragraph in which these are set forth ('97 page 35). "The animal and vegetal poles are considered the fixed points in the egg. In the ectoblast the stem or parent cell is in all cases the upper

one. The stem cell in the entoblast and mesoblast is in every case the lower one. If, in any case, the cleavage is meridional, (an exceedingly rare thing) the right moiety is considered the stem cell. The terms right and left are employed in the usual sense, i.e., right is clockwise left is anti-clockwise. A cleavage is oblique to the right or following Lillie ('95) dextrotropic, when the upper moiety lies to the right of the lower; it is oblique to the left, or leiotropic, when the upper moiety lies to the left of the lower".

THE UNSEGMENTED OVUM.

The ovum of *I. magdalenensis* varies in color from a light pink to pinkish gray. It is perfectly spherical, and measures exclusive of the chorion 0.4 m.m. in diameter. It is densely packed with a finely granular yolk, that renders it perfectly opaque, making observations very difficult on the living egg. The protoplasmic portion at the animal pole is very slightly developed and is to be determined by sections only. The cleavage nucleus is slightly eccentric lying on the side toward the animal pole, and is characterized by a finely granular chromatin

network suspended in a relatively abundant achromatic substance. This feature of the nucleus is the same for all cells for a considerable period of development and it often renders it difficult if not impossible to follow some of the phases of development of the entodermal cells, a difficulty that is not lessened by the abundance of yolk.

Two polar bodies are formed and the chromatin of the first one occasionally redivides though in no case have I seen the division affect the cytoplasm. The chromatin consists of densely aggregated irregular knots, staining intensely, while the cytoplasm is perfectly transparent and is little if at all stained by logwood dyes. In stripping the chorion from these eggs the polar bodies are usually dislodged, although normally they persist at least until the 160 cell stage.

Each egg is enclosed in a chorion bristling with many fork-like processes (fig. ^I), almost exactly identical with those figured by Kowalevski for *Chiton Polii*. It is much thinner than the chorion of *Mopalia lignosa* or *Katharina tunicata* whose eggs are laid singly, but the albumen in which the eggs are embedded no doubt acts as

an additional protective envelope.

I have taken up the study of the oogenesis only in a general way, but there seems little doubt but that

observations on the formations of the chorion are correct. The chorion is accordingly the metamorphosed follicular epithelium, and not a product of the egg itself. On the other hand a delicate vitelline membrane is present in the fertilized egg and also, I believe, in the unfertilized ones.

The individuals that laid in the aquarium were brought from a point three or four miles distant from the laboratory. In their transportation the water was changed several times. The eggs subsequently laid in the aquarium, with relatively few exceptions, did not develop, from which I conclude that the sperm were in the sea water and were removed in changing it, whence it probably follows fertilization takes place outside of the body. 1.

This method of fertilization is described by Metcalf for

-
1. The eggs that were fertilized developed normally, proving that the environment was normal.

Chiton mormoratus and *C. squamosus*, and I have noticed it in the case of *Mopalia lignosa*.

Some of the specimens were killed while laying, and the eggs in the ovary showed the first maturation spindle in process of formation. It is surrounded by a small protoplasmic area while the remainder of the egg is uniformly filled with yolk. The spindle is of very small size, being but about one-ninth the diameter of the egg.

First cleavage, 2 cells.

Basing my observations on those chiton that laid in the aquarium, one hour and ten minutes elapses from first fertilization to the first cleavage. After this period a slight flattening appears at the animal pole which is followed by the formation of a furrow rapidly encircling the egg. This divided the ovum in about ten minutes. At this time the two blastomeres are somewhat spherical but they soon become so pressed together that they are almost perfect hemispheres: indeed in the living egg it is often difficult to make out the boundary between the two. By this division two cells result, one of which is larger than the other, but this difference in size is usually

very slight, and often it is impossible to detect any difference.

I have taken much pains to attempt to determine whether this inequality in the masses of these blastomeres is correlated with the more rapid development of the posterior quadrants, but I have found no constant feature which would enable me to orient the egg in these early stages. That an early differentiation occurs appears probable, but the landmarks up to the 32 cell stage are of too uncertain a nature to give any definite answer to the question, and hence I am unable to state that this larger cell contains the material of the 1st and 2nd somatoblasts as in *Nereis*, *Unio*, *Amphitrite* etc. The spindle that initiated this cleavage is fully half of the diameter of the egg in length: while the one giving rise to the first polar body is one-ninth the egg's diameter.

Second cleavage-4 cells.

In about half an hour the second cleavage furrow appears at right angles to the first and behaving in about the same manner divided the egg into four cells. Usually one of these products is slightly larger than the

others, and in the drawings I have oriented it so that it corresponds to D in many other forms. (Nereis, Unio, Crepidula etc.) but, as I have said, no definite proof is at present forthcoming that such is the case.

A cross furrow appears that is especially well marked at the animal pole, and on the vegetative pole one is present at right angles to the first, but the cells forming it are simply in gentle contact. This feature persists for a considerable period in many eggs, affording an excellent means of orienting the blastomeres and relating the first and second cleavage planes to the axes of the embryo. In Ishnokinon, however, in the transition from the four to the eight cell stage it ceases to be definite enough to be used as a landmark, and hence I am ignorant of the relation of the four cell embryo to the adult.

I have not taken up the minuter processes of cell division, but one matter calls for remark. Previous to the division of the cell I have often noticed that the two centrosomes come to lie on opposite sides of the nucleus, and the fibres radiating from them often indent

the nuclear membrane as in Figs. The process is very similar to that first figured by Watasé ('90) in his study of the cephalopod cleavage, and it appears to be characteristic of the mollusca in general.

Third cleavage-8 cells.

The next division gives rise, in a dextrotropic direction to four cells, the first quartette of ectomeres. The spindles which introduce this cleavage have their upper ends inclined to the right, and usually a distinct bulging of the upper right hand side of the cells occurs before the division is completed. When the blastomeres are formed they will be seen to lie some distance to the right of the parent cells,¹ and therefore partly over the furrows between the macromeres. This shifting occurs principally before the complete division of the cells although in many cases the halves of the central spindle in the daughter and parent cell are slightly bent showing that a shifting has occurred after the cells are formed. But

1. In the tables of cleavage I have rigidly adhered to the rules given on page ,but throughout the descriptions I have used the term parent or stem cell in a looser sense, designating it as the larger of the two products of a cleavage.

this latter stage of the rotation is almost imperceptible and is not unlike numerous examples which may be noted throughout embryonic development.

Fourth cleavage-16cells.

In accordance with the law of the alternation of cleaveages as formulated by Kofoed ('95) this stage is introduced by a leiotropic division of the first quartette which forms four cells of equal size, termed by Wilson the trochoblasts. Usually at the same time the macromeres cleave in a leiotropic direction forming the second quartette of ectomeres. The spindles giving rise to the trochoblasts and to the second quartette 2a, 2b etc. are almost in line with each other and consequently when the cells are completely formed they lie pressed together between the macromeres on one side and the stem cell of the first quartette on the other.

Since the advent of Professor Wilson's beautiful study of Nereis in which for the first time the origin of the prototroch was accurately determined, a number of papers have appeared which greatly advance our knowledge of this organ in other forms. Among annelids there is

Amphitrite, Clymenella (Mead), Podarke (Treadwell), and Arenicola (Child), and in Gasteropods four species of Crepidula (Conklin) in which the accurate development of the prototroch is known. And also there are many forms including annelids, gasteropods, polyclades, and chitons, in which there is an essential similarity of origin and behavior of like cells in the early stages. As their later history has not been determined nothing definite may be claimed, yet it appears altogether probable that in this respect they do not differ from the above accurately studied forms. At all events this fact remains, that among gasteropods, annelids and chitons in which the cell lineage has been carefully followed the prototroch is in part formed from the first division of the first quartette. Conklin has already emphasized the fact that there is almost certainly a homology between the trochoblasts of gasteropods and annelids, and the development of the prototroch of Ishnochiton gives greater strength to the view. In fact throughout the development, not only in the behavior of the trochoblasts, but of other cells, we have many striking resemblances that argue strongly for a gen-

etic relationship between annelids, gastropods and chitons.

In Nereis, according to Wilson, not all the substance of the trochoblasts enters into the formation of the prototroch. On the other hand Mead finds that it does in Amphitrite and Clymenella, as has Treadwell for Podarke and Child for Arenicola. In gastropods Conklin finds that the two anterior groups of trochoblasts enter into the velum and possibly this may be said of the posterior also. In Ishnochiton the entire substance aids in the formation of the prototroch, but they are not the only locomotor
1.
cells from the first quartette.

Kowalevski ('83) in his figs. 9 and 10 of the development of Chiton Polii shows the sixteen cell stage very similar to figs. of Ishnochiton. Without doubt he represents the trochoblasts and their parent cells, second quartette and macromeres. In the transition from the eight to the sixteen cell stage he determined

1. In accordance with Mead I shall term the cells forming from the first quartette, and corresponding to the trochoblasts of annelids, the primary trochoblasts.

the origin of the second quartette, but was unable to decide as to the origin of the cells (trochoblasts). However, it appeared more probable in the rhythm of segmentation that they arose by the division of the cells corresponding to the first quartette, a view which is undoubtedly correct.

Metcalf () represents a sixteen cell stage in the development of *Chiton squamosus* and *C. marmoratus* in which the second quartette forms in the usual manner, but the cell which forms as the first product of the first quartette arises in a dextrotropic direction which is the reverse of what we find in polyclades, annelids and dextrally twisted gasteropods.¹

Regarding the second quartette cells little remains

1. Professor Metcalf kindly informs me that his conclusion was based on a few eggs which showed a distinct bulging on the lower left hand side of the first quartette cells. The eggs were too opaque to allow the spindles to be seen and therefore this bulging was considered to be an indication of division. Consequently it seems to me quite probable that these cells in their formation do not form an exception to the alternation of cleaveages.

to be said at this point; all are of essentially the same size, the one giving rise to the first somatoblast being no larger than the rest. Their history will be more fully considered in special sections. At present it may be said that they are of great importance in the development of the embryo and also that their destinies are most diverse, entering as they do into the formation of the sub-umbrella ectoderm, the stomodaeum, the shell, foot and probably the nervous system.

Fifth cleavage-24 cells.

In this cleavage also there are eight new cells formed, four from a cleavage of the large first quartette micromeres wherein the newly formed products lie radially disposed about the animal pole; and four from a division of the macromeres giving rise to the third quartette. The first cells mentioned arise in a dextrotropic division and constitute the apicals. These are relatively smaller than the corresponding cells in annelids and molluscs and their parent cells, the intermediate girdle cells of annelids, are much larger than in any other form. In certain specimens the apical series sinks below the general surface,

and in some cases when greatly crowded by the large surrounding parent cells they disappear almost entirely from view, but at or before their next division they commence to emerge again, and ultimately become level with the other cells of the upper hemisphere. It reminds one of similar movements in the rosette series of annelids, and of the so-called invagination at the animal pole of Neritina.

As just said, the cells corresponding to the intermediate girdle cells are relatively very large, while in annelids they are of the same size or smaller than the apicals to which they give rise, and, as will appear later, it is partly owing to this fact that the cross as it appears in gasteropods is not forthcoming in the same cells in annelids.

Up to this time three quartettes have separated from the macromeres: the cells of the first and second are of about the same size; those of the third considerably larger than either; and the macromeres are probably no larger than either of the first two. All contain yolk in about the same relative quantity, and for this reason

the free surfaces of the cells always stand out round and full, in marked contrast to the protoplasmic cells of this same stage where they are closely appressed against the huge yolk-laden macromeres.

Sixth cleavage-36 cells.

The primary trochoblasts were formed at practically the same time as the second quartette and in this cleavage both sets of cells divide at almost the same time. The former segments dextrotropically, the two resulting cells occupying the same position as did the parent cell. The second quartette also divided dextrotropically into a lower larger and upper smaller cell, which group also retains the original position of the mother cell, though at times a slight shifting to the right occurs in the upper product.

About this time the parent cells of the trochoblasts and the apical series, (), are seen to be in the process of division. For some time I looked upon them as the cells corresponding to the basal cells of the molluscan cross (1a^{1.2} etc. of *Crepidula*), and hence considered that this division would result, as in *Crepidula*, in the

formation of the median and basal cross cells proper, but a more careful study has shown this to be incorrect for the cells that form at this division are true trochoblasts, the second set formed from the first quartette. These cells have formed by a leiotropic division and lie closely wedged in between the parent cell and the two trochoblasts of each quadrant. The first quartette of ectomeres therefore gives rise to the primary trochoblasts, and this third division produces cells which I shall term the accessory trochoblasts. Strictly speaking these accessory cells are just as much to be considered primary as are those produced at the first division of the first quartette, but I shall let this be tacitly understood and therefore speak of them simply as accessory trochoblasts. Also I have used the term accessory in view of the fact that these cells form but half the number of cells produced by the primary trochoblasts, that is to say, the primary trochoblasts give rise to four cells in each quadrant while the accessory form two. These latter cells appear from the start similar to the primary trochoblasts, and throughout their development

they are alike in all essential regards.

The fuller significance of this cleavage will be considered in the section on the prototroch, though it may be added in this connection that the parent cells of the accessory trochoblasts are now to be considered as the basal cells of the cross since in their future history they bear a remarkable likeness to the cross cells in the gasteropod.

Seventh cleavage-60 cells.

About this time a leiotropic division occurs in each cell of the third quartette by which four cells are formed about one third the size of the parent cell. Immediately after their formation they show most clearly their leiotropic position, crowding onto the territory of the second quartette and macromere of the next quadrant, but very rapidly they adjust themselves symmetrically in the angles between the macromeres. (compare figs. and) These cells form by far the greater part of the stomodaeum, and following Wilson I shall term them stomatoblasts. This designation in the case of *Nereis* however was applied to second quartette cells which in *Ishnochiton* also become stomatoblasts, and in view of this fact

I have designated both sets of cells stomatoblasts, speaking of them either as second or third quartette stomatoblasts as the case may be. All these third quartette stomatoblasts appear of about the same size, although later it will be noted that the size of the two posterior exceeds that of the anterior, which aids very materially in bringing about marked changes in the form of the embryo.

Division of apicals. The 44 cell stage is ushered in by the division of the apicals. In some cases the spindles are almost radial, but the division is invariably leiotropic. The area of the "rosette series" (etc.) is thus increased but its relative position remains the same, and this is true as far as its history has been traced, the tips of its arms always remaining in contact with the accessory trochoblasts.

The next division affects the upper cell of the second quartette. By a leiotropic cleavage it divides into a triangular cell lying to the right and somewhat above the remaining narrow rectangular one. The upper cell is the tip cell of the cross, and is a secondary

trochoblast of Mead-a term which I shall adopt. The remaining cell also in Amphitrite forms two cells one of which enters the functional prototroch. This latter feature does not appear in Ishnochiton, though it will be seen that the history of this cell is very intimately bound up with that of the prototroch. It enters into the formation of a "supporting layer" of the velum, a third row of cells lying below the two upper functional prototrochal rows.¹ I am not certain that this lower layer becomes ciliated as the movements of the huge velar cilia prevent all observation in a living state. If cilia be present they must be very delicate, and in preserved specimens the killing agents give results too uncertain to be relied upon.

The following division forms the basal cell proper of the molluscan cross and the median cell. The spindle is perfectly radial and all the divisions occur, with slight irregularities, at the same time, and the products formed are similar in each quadrant, there being now no

1. Cells having the same origin and much the same relations arise in Nereis, according to Wilson, and are designated the post-trochal cells, which will receive attention in the consideration of the development of the second quartette.

pecularity of the posterior arm as in *Crepidula*. At this stage therefore the cross is perfectly symmetrical with three cells in each arm which lie in the antero-posterior axis and 90° removed.

Each of the primary trochoblasts cleaves simultaneously giving rise to four cells in each quadrant. The direction of the cleavage is leiotropic and there is little subsequent shifting. This is the last division that ever occurs in these cells.

Post trochal cell (). In all but the posterior quadrant the cells cleave in a leiotropic direction to form a cell which like enters into the formation of the supporting layer of the velum. These cells are in very intimate relation with the primary trochoblasts, and at the time of their formation give evidence of entering the velum but as the tip cells place themselves in their final position they become situated below the prototroch forming a third row.

Eighth cleavage-64 cells.

Posterior 2nd. quartette stomatoblast. The next cleavage in which the posterior second quartette stomat-

oblast () forms (fig.) is one of the most interesting in the entire cell lineage. As the figure shows, the division is leiotropic, and results in a smaller lower cell in contact with the mesoblast. In this connection it may be well to state that in being leiotropic this cleavage conforms to the spiral type; in the other quadrants however the cleavage producing the stomatoblasts is perfectly horizontal, thereby conforming to the bilateral condition.

The peculiarities of this cleavage will appear to better advantage if we compare the second quartette products of fig. with those of fig. . In this we are comparing the right quadrant with the posterior at a time when the number of cells is the same in each. The post trochal cells are homologous, and the tip cell in the right quadrant when the division is completed will be similar to the other, hence in the further study of this quartette these cells will not be considered.

The stomatoblasts are in contact with the macromeres in each and when the division of is completed the relation of the cells will be the same in both, although there are some differences in size. But while

the appearances are the same the cells which appear homologous have not the same designation. But if we compare two quadrants in the same egg at a somewhat earlier stage (figs. and) the cause of this phenomenon will be understood. Neglecting the tip cells and post trochal cells which have the same origin it will be seen that the remaining cell in fig. has by division given rise to the upper post trochal cell () while in fig. it has produced the stomatoblast, the cell corresponding to the left post trochal arising at the next cleavage; also in the other case the stomatoblast arises at the next division of

In considering the manner in which this anachronism arose it will be noticed that in each case the stomatoblast arises at the time when the fourth quartette is forming. Secondary changes have brought about an acceleration of the cleavage of D (formation of mesoblast) but despite this fact the stomatoblast arises at the same relative time as in the other quadrants. This acceleration in the formation of the mesoblast does not bring about an increase in the rapidity of cell divisions in the

first somatoblast and hence if the somatoblast arise at the time when the mesoblast forms, the cleavage which originates the left post trochal cell must be omitted.

Thus it seems to be of more importance that the stomatoblast be formed at this time than that the post trochal cell be produced. In other words, the correlation between the stomatoblast and the mesoblast is closer and perhaps of more vital importance than that existing between the post trochal cell and the prototroch. It clearly appears to an onlooker that if the post trochal cell arose at the same time in all quadrants, the stomatoblast arising at the next cleavage would not be able to relate itself intimately to the mesoblast which has already retreated to a considerable extent within the embryo.

I have endeavored to ascertain what effect this anachronism has upon the future history of the cell. In this case there is no evidence to be relied upon; the cell never gives any indication of being a post trochal cell, and neither do the tip cell nor right post trochal cell which have arisen normally. Thus it becomes impossible to differentiate the result of precocious segregation from those due to anachronism.

In the other case in which I believe an anachronism has resulted, delaying the formation of the accessory trochoblasts in *Crepidula* (page) two cells are in the later development thrown away. These products may contain the substance of the accessory trochoblasts, but how much their degeneration may be due to anachronism and how much to other agencies it is impossible to determine.

These two isolated cases afford very little data for an understanding of the significance of such cleavages. However they serve to show in the clearest manner that in the radial condition the blastomeres are differentiated, that they are of morphological importance, that cells are not what they are by virtue of their position, and that a very close correlation and interaction exists between various cells of the same group which extends into other embryonic layers.

As development proceeds and the general ground plan of the embryo has been developed the correlation of the germ layers is apparently not so close. Roux and O. Hertwig have shown for example that with reference to the same stage in the differentiation of the medullary

canal of the frog, the entoblast and mesoblast may produce the same structures at different times, and in Ishnochiton certain cell cleavages and shifting of cells may occur at different times with respect to the closure of the blastopore or its later migration. However in Ishnochiton it is very difficult to judge of the extent of the correlation as the form of the various layers or organs is not a safe criterion for determining the amount of differentiation which is present.

Ninth cleavage-72 cells.

Division of 3rd. quartette . The upper products of the third quartette are the next cells to divide. In each the spindle is perfectly horizontal and the resulting cleavage produces two cells of equal size. This is the first of a series of cell divisions in which the spindles inaugurating the division lie at right angles to those of the preceeding cleavage.

The spindles form in the posterior quadrants, and the cell divisions occur, slightly in advance of those of the anterior (fig).

First division of the accessory trochoblasts. The

only division which the accessory trochoblasts undergo occurs at this stage. The spindles arise simultaneously, and placing themselves in a leiotropic position, divide each cell in two equal halves. The primary trochoblasts now number six in each quadrant, and with the exception of an increased external surface, they occupy the same position as at their formation.

Tenth cleavage-73 cells.

Mesoblast. The mesoblast arises from the posterior macromere by a cleavage in which the spindle is usually slightly leiotropic-though in many cases it assumes a perfectly radial position. As a result of the division usually by far the greater part of the substance of the macromere enters the mesoblast, and the macromere is often scarcely larger than the full formed mesoblast nucleus. In other cases the macromere is a relatively long and slender cell, extending for a considerable distance into the egg.

At the time when the mesoblast forms it becomes shifted, in some unaccountable way, slightly to the right: the third quartette stomatoblast () becomes somewhat

smaller superficially and the outer border of becomes lengthened. Later a perfectly symmetrical condition arises which continues henceforth.

In this case it is most obvious that the mesoblast is modified from the entoderm. It arises a little earlier than the other members of the fourth quartette and is comparatively larger, but the general features of its origin and position are unmistakable.

Mead urges that the mesoblast arises at the ideal 64 cell stage, and while this appears to be correct many modifications exist which destroy this typical condition. In such forms as *Crepidula* the ectoblast is slow in its development, and the mesoblast arises at the 25 cell stage and all conditions exist between this extreme and *Ishnochiton* where the mesoblast arises as the 73rd. cell. These modifications have probably arisen from various causes, such as the accumulation of yolk and precocious segregation, but the most remarkable fact of it all is that despite these acquired characters the mesoblast in annelids and molluscs and possibly flatworms arises at the fourth division of the posterior macromere.

In this connection it is important to note that in Ishnoochiton there is another addition to the rapidly increasing list of forms in which the main structural details of development are similar. Among annelids, flatworms and molluscs, save cephalopods, the first three quartettes of cells gives rise to the entire future ectoblast; and at the next division of the left posterior macromere the primary mesoblast arises; while the remaining cells of the fourth quartette and the macromeres form entoblast. And these remarkable resemblances do not cease at this point but continue more or less clearly defined as far as the development may be followed. Their fuller significance therefore will be considered when these later phases have been described and the evidence is all in.

Also the form of the future embryo and larva are at this time outlined, the protoblasts of all the definite regions and organs of the body being present. This is shown in the following table which is here introduced that it may render more intelligible the relation of the various quartettes and the part they play in the devel-

opment, a history to which the following pages are devoted.

History of the first Quartette.

As will be remembered the first quartette of ecto-meres gives rise to the head vesicle, the apical sense organ, cerebral ganglia, and a portion of the prototroch. There are no head kidneys.

Conklin ('97) has shown in *Crepidula* that some of the products of the first quartette (part of the posterior arm of the cross) pass down into the posterior hemisphere. Mead finds the same thing to be true in *Amphitrite* though probably to a less extent. In *Ishnochiton*, on the other hand, cells of the first quartette becoming ciliated aid in filling up the posterior gap in the prototroch but none pass below it. Hence in a consideration of this quartette, we deal with the anterior hemisphere whose lower boundary is the posterior border of the prototroch.

For purposes of convenience the tip cells of the cross since they enter into the formation of the velum will be considered in this connection though it should be borne in mind they are second quartette cells.

Trochoblasts and Tip cells-formation of Prototroch.

To recapitulate briefly, the first division of the

first quartette gives rise, leiotropically, to the primary trochoblasts. (fig.). These are formed at the same time as the second quartette and with these latter cells alternate about the equator of the embryo. The next division affecting these blastomeres occurs in the 32 cell stage when each cell cleaves dextiotropically into two products of equal size. Almost immediately after this cleavage each of the four cells of the first quartette corresponding to the intermediate girdle cells of annelids (

) divides in a leiotropic direction giving rise to a cell which lies to the left of the two primary trochoblasts of each quadrant (fig.). Of about the same size as either primary trochoblast it also subsequently undergoes similar changes and finally enters the primary protroch. For this reason I have designated them the accessory trochoblasts. Each of the primary trochoblasts at the 52 cell stage divide in a leiotropic direction giving rise to a group of four cells in each quadrant occupying about the same position held by the parent cells. Somewhat later the accessory cells divide in a dextiotropic direction forming two cells in each quadrant. These co-

cupy the space between their parent cells () which now correspond to the basal cells of the cross in gastropods, and the primary trochoblasts.

Meanwhile changes are going on in the second quartette by means of which certain cells are formed that also enter into the formation of the functional prototroch. When the embryo consists of 30 blastomeres each of the cells of the second quartette by a dextrotropic division cleaves into two cells, the upper right hand one in the 44 cell stage dividing again forms the tip cell of the cross.

In quadrants A, B and C each of these tip cells divides by an almost meridional cleavage into two equal products which ultimately becoming ciliated enter into the functional velum. In quadrant D on the other hand the same division of the tip cell occurs, yet the cells, before the prototroch becomes functional, are drawn below the level of that organ, and losing their original function they enter into the formation of the ventral plate. In this way a posterior gap occurs similar in all respects to the prototrochal gap of annelids.

All the cells of the prototroch are now formed and comprise eight accessory, sixteen primary and six secondary trochoblasts, thirty cells in all. As will be seen two cells, products of the median cell of the cross, becoming ciliated aid in filling the posterior gap, but their function is secondarily acquired, and ought not, strictly speaking to be included in the primitive velum.

In the incipient stages of its formation the prototroch consists of an angular band of cells encircling the embryo. The tip cells lie below the level of the future velum, and the remaining cells are somewhat also out of line; all the cells are bold and round of outline and their exposed surfaces are apparently greater than at any period until their degeneration sets in. Nevertheless very shortly after its formation, in fact even before it is fully formed, it commences to assume those features which characterize the later stages of its history, and certain movements of a migratory character are met with among the cells which work out decided changes in the relation and position of the velum. Attention has already been called to the fact that when the tip cells are form-

ed each is in contact with the trochoblasts of the first quartette of adjacent quadrants, and consequently the groups of cells which are to enter into the formation of the prototroch form a ring about the embryo broken at one point on its posterior side. During their subsequent division these groups maintain essentially the same position, and when every cell that is to enter into the formation of the velum is present the relations are practically unchanged. But in the functional condition the prototrochal cells form two rows encircling the embryo, while in diagram or fig. the prototroch is two cells wide as regards the first quartette trochoblasts but only one row wide in the secondary (tip cells). The method by which the completed condition arises is most interesting and affords a phase of cell dynamics that is not easily explained by simple mechanical principles.

In diagram or fig. it will be seen that a cell of the present lower row is in contact with the right tip cell and consequently belong to the upper row while and will come to lie in contact with the right and left tip cells respectively which are situated

in the lower row. The movements are not brought about by a simple shifting of the cells in question alone, but is a more profound movement that extends itself into other sets of cells.

First, regarding the method by which and come in contact. In their early stages these cells are cuboidal in shape (fig.) but shortly after the division to form the tip cells they will be seen to become somewhat elongated on the side next the tip cells and their angles on that side will become more and more acute (compare figs. and). In other words they become wedge-shaped with the pointed ends tending to come in contact with each other. This movement and change of shape becomes more marked, and the cells seemingly pry apart the cells of the first quartette (some median cells of the cross) and the tip cells lying below them. Generally this change of shape affects the entire cell, but frequently the wedge-like portion includes but half the cell, the remaining half appearing as a more or less globular mass apparently more passive. The appearance may be compared to an amoeba with one pseudopodium which, pushing out, ultimately

comes in contact with a like process from a neighboring cell. Some specimens show the processes to be extremely slender, sometimes requiring careful focusing to observe them, but after the two cells come in contact they gradually assume a rectangular form which they retain throughout development. By this means spaces nearly or quite as wide as the upper border of the tip cells are bridged in three quadrants. The upper row of the velum is now complete, and consists throughout of first quartette trochoblasts.

In the meantime changes have occurred which produce a continuous lower row in which and are in contact with the tip cells. In the earlier stages these latter cells are isolated from any of the lower row, but two series of movements bring about the final continuity. The most important are the changes undergone by the tip cells. As a general thing they are more or less triangular with their bases in contact, and their apices directed outward toward the cells of the lower row. They always remain connected in this manner, but as the embryo develops the bases become smaller and the altitude great-

er by which means the apices lie much nearer the cells and . This will be made more clear by a comparison of . At the same time a movement generally occurs in the lower row of primary trochoblasts by which the cells become more or less rectangular. Frequently the extremities of and lying next to the secondary trochoblasts become pointed and their apices ultimately meet the advancing tip cells. At first the contact surface between these cells is small, but in a short time it grows broader and becomes as great as the smaller diameter of the cells. Thus by movements of the tip cells and primary trochoblasts the lower row becomes complete.

In the second quartette of the posterior quadrant in the early stages the divisions undergone are much the same as those of the other quadrants, but generally commencing about the time of the division of the tip cell changes occur which cause these latter cells to lie below the line of the prototroch and consequently a gap is produced as wide as the original tip cell.

The movements whereby the upper row of cells is completed are similar to those of the remaining quadrants.

They generally extend themselves across the gap between the upper cells of the somatic plate and the median cross cells lying above and ultimately come in contact. The contact surface however always remains of smaller area than in the other cases.

The lower row cells all become flattened from above downward, increasing their long diameters, and the cells and assume a wedge-shaped form similar to that of the cells of the upper row. They come in contact with the tip cells, but as these gradually sink into the lower hemisphere they continue to flatten and the apex to advance across their upper surfaces, and gradually, after they have increased to nearly twice their original length, they meet in the median dorsal line. Generally the processes in this case are very slender, and they usually remain in this condition permanently, so that the contact surface is as slight as in the case of the cells of the upper row bridging the gap. Were this condition of affairs to continue, the prototroch would be a very feeble structure posteriorly, but it becomes reinforced by two first quartette cells () that are derived from the me-

dian cell of the cross. The position of these cells may be seen in diagram or fig. . As a general thing each cell divides and the resulting products arrange themselves in such a way that the prototrochal band is as wide here as in any other part of its course.

When first formed the trochoblasts apparently differ in no way from the remaining cells of the ectoblast, but as development proceeds and they commence to assume their permanent positions they become characterized by features that are found in no other cells of the embryo. Outwardly these changes manifest themselves by a flattening of the external surface which takes place to such an extent that at the time when the cells become ciliated they form a constriction about the egg and appear in marked contrast to the remaining ectodermal cells. During this time the trochoblasts have decreased their external, and in many cases increased their internal, surfaces. In other cases the inner and outer surfaces are about equal, and at such times the cell appears barrel shaped in section.

In the interior of the cells after the cilia have

become active the cytoplasm shows imbedded in it droplets of various sizes that are probably metabolic products formed as a result of the activity of the cilia. These continue to accumulate for several days, until the fluid contents is in excess of the cytoplasmic and at the free swimming stage the cells become turgid and project above the general surface of the body.(fig.). About two days after the embryo has escaped from the chorion, the cells, which have been gradually pushed out, burst, and are thrown away. For several hours a slight constriction appears where the velum was located, but after that time all traces of the organ disappear.

Comparisons.

Among molluscs the origin of the velum has not hitherto been accurately determined, but in two gastropods, *Meritina*, and especially *Crepidula*, several facts are known which are of the highest interest.

In the first form mentioned, Blochmann determined that the right and left tip cells enter the velum. In thus becoming secondary trochoblasts they correspond to mollusca, and to several annelids also, but how much far-

ther the resemblance may extend cannot at present be determined, since the development of the remaining portions of the velum is unknown.

In *Crepidula*, on the other hand, much more is known. The two anterior trochoblasts, formed at the first division of the first quartette, become velar cells. Whether the posterior cells are destined to a similar fate has not been determined, but since there is a wide posterior gap in the velum it may be that these cells remain non-functional.

Anteriorly, certain cells from the second quartette, derived probably from the tip cell, enter the velum, and there is a strong probability that the tip cells of the transverse arms also contribute. These velar cells divide repeatedly, and ultimately become relatively small, yet it is obvious that the portion of the velum just described has probably the same origin as in *Ishnochiton* and, as will be shown, in the annelids.

The annelid prototroch consists of a simple band of cells encircling the embryo, and is composed of relatively few cells, whose origin has been determined in several

cases. There is a general correspondence throughout, but some modifications have been described whereby the development departs from the general plan. For example, Wilson shows that the prototroch of *Nereis* arises wholly from the primary trochoblasts, twelve of the sixteen cells from this source becoming functional. The second quartette accordingly contributes nothing to this structure, although some of its products become post trochal cells lying below the locomotor organ. This prototroch, and possibly that of Hydroïdes, in which there are eight cells in the prototroch (Wilson) is much simpler than is ordinarily found in annelids. And as has been noted, all its cells arise from the primary trochoblasts and therefore from the same cells which enter the velum in *Ishnochiton*.

In *Amphitrite* and *Clymenella*, Mead shows that the primary trochoblasts give rise to sixteen cells all of which enter the functional prototroch, and the same fact has been proved to be true in *Podarke* (Treadwell), and in *Arenicola* (Chilod). The origin, development and fate of these cells is precisely similar to the primary trochoblasts in *Ishnochiton*.

The second quartette in *Amphitrite*, *Clymenella* and

Arenicola furnishes three cells in each quadrant, except the posterior which enter the prototroch. Two of the three are homologues of the divided tip cell in Ishnouchiton which are sometimes secondary trochoblasts while the third corresponds to of the post trochal cells. It is to be noted however, that while the second quartette supplies a cell which is non-functional in Ishnouchiton, this latter form furnishes an accessory trochoblast whose homologue is non-functional in the annelids. The remarkable fact now presents itself, twenty-two of the twenty-five prototrochal cells in Amphitrite, Clymenella, Arenicola, and possibly Podarke are exactly homologous with the velar cells in Ishnouchiton. A more complete resemblance it would be difficult to find and I see no other alternative than that it is an indication of a close genetic relationship between the molluscs and the annelids.

Ectoplastic Cross (so called Molluscan Cross).

Briefly recapitulating, the first division of the first quartette of ectomeres gives rise to the primary trochoblasts; the second forms an inner set of four cells

lying at the animal pole-the apicals, while the parent cells are spoken of in gasteropods as the basal cells of the cross. As regards the basals however, it will be seen that this terminology will not answer in the case of *Ishnochiton*, since at their next division they do not give rise to cross cells, but to the accessory trochoblasts. After forming these cells, the parent cells in position and subsequent history answer to the basal cells of the molluscan cross.

At about the time when the accessory trochoblasts are formed the upper of the two cells of the second quartette in each quadrant divides, forming the tip cells of the cross (). The cross in *Ishnochiton* therefore is composed at this stage of twelve cells, four apicals, four basals, and four tip cells. In the 60 cell stage each basal divides equatorially into an inner and outer cell, the basal proper and the median cell of the cross (fig.). The arms of the cross are alike in each quadrant and taper from centre to tip, a condition of affairs that is not disturbed by the subsequent radial division of the basal cells.

In the meantime the four apicals have divided into eight cells, of which four lying symmetrically about the animal pole constitute the apical rosette, while the four peripheral cells are termed the peripheral rosette. This rosette series also forms a cross whose arms lie midway between the arms of the molluscan cross. The two crosses have hitherto been considered together in molluscs, but in *Ishnochiton* as the arms of the molluscan cross become dim and indistinct in outline the rosette series comes into prominence as a distinct and beautifully symmetrical cross whose arms lie wholly in the velar field. The history of these two crosses are so widely different that it seems best to consider them separately in their later stages.

Axial Relations. The cross when formed is distinctly dextrotropic, but this condition is somewhat modified by the formation of the peripheral rosette when the arms of the cross become more radial in position. The tip cells of the cross however usually continue to lie somewhat to the left of the basal cells, but when the primary trochoblasts cleave the second time forming four cells in

each quadrant, the tip cells assume a radial arrangement.

This continues through the cleavage of the basal cells up to the time of the division of the medians.

In the dextrotropic stage of the cross the long axis of the arms does not coincide with the antero-posterior axis nor with the corresponding one passing through the right and left halves of the body. The tip cells lie in these axes but the major portion of the arms lies to the right in each quadrant. But later the radial condition is assumed and the arms become anterior, posterior, right and left, which position they probably permanently retain.

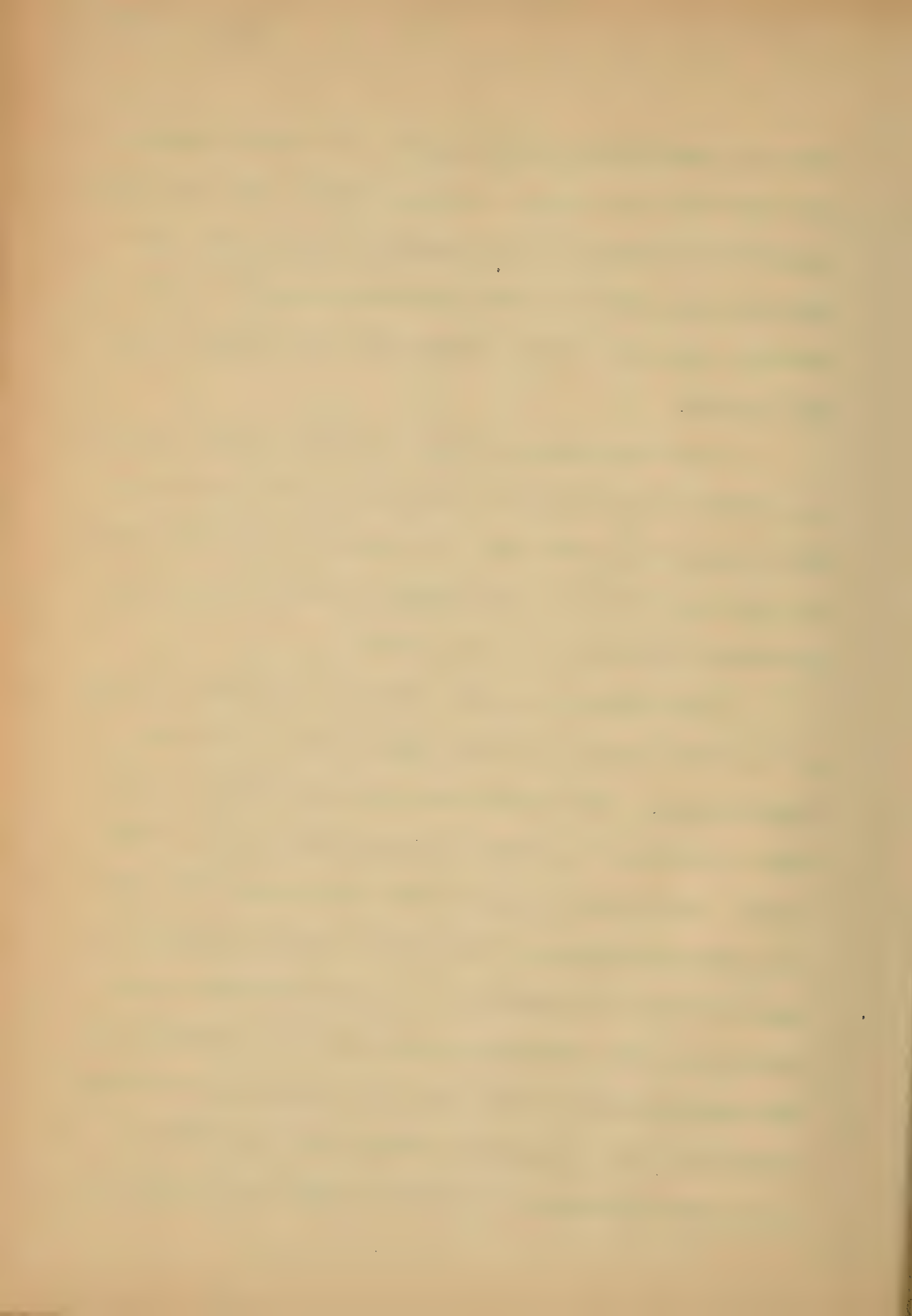
Later History.

Among the few gasteropods which have been accurately studied a cross is shown to be present and in general appearance, position and history as far as this has been traced it is similar to the chiton cross and yet the two structures are not homologous. In what does the essential difference consist? In attempting to answer this question it may be well to note that Ischnochiton throughout its larval development shows a more primitive character than the annelids or gasteropods whose cell lineage has been carefully studied; and that its development is more closely related to the annelids than to the gasteropods.

Hence in considering the differences that exist between the gastropod and chiton crosses we shall note first the condition of affairs in the annelids, and in this light consider what obtains in the gastropods, and then if possible bring the various structures into harmony with one another.

In the first place it must be borne in mind that in *Ishnochiton* there are three divisions of the first quartette before the basal cell is formed, and that this latter cell is formed by a leiotropic division in which the accessory trochoblast is also formed.

In *Amphitrite*, as shown by Head, the first division of the first quartette of ectomeres gives rise to the trochoblasts. These cells are relatively larger than those in chiton, appearing fully as large as the parent cells. The second division forms the apicals which are also relatively larger than what we find in *Ishnochiton*. Hence between the formation of the trochoblasts and the apicals the four remaining cells, called in annelids the intermediate girdle cells, are the smallest of the first quartette. This is clearly brought out in diagram () of the 32 cell stage and also by comparing it with the



same stage in *Ishnochiton* (fig.). In the diagram, the trochoblasts have divided and either product is nearly or fully as large as the intermediate cell; and this latter is invariably smaller than the apical cell of the same quadrant. In *Ishnochiton*, on the other hand, either of the two trochoblasts in each quadrant is about the same size as an apical cell, and the cell with the same designation as the intermediate cell is nearly or quite as large as the two trochoblasts and the apical taken together. But great as these differences are, there is essentially the same arrangement of cells in each case; the apicals lie at the animal pole, and in the angles between them lie the intermediate cells, while at their outer extremities lie the trochoblasts; also in both, the intermediate cells touch the second quartette. The intermediate cell now cleaves leiotropically, forming a cell in each quadrant that lies in the gap between the groups of trochoblasts. It is this cell in *Ishnochiton* which forms the accessory trochoblast and it is important to note that the division forming each is leiotropic.

In the diagram of *Ishnochiton* the accessory troch-



oblasts are comparatively small cells, lying one in each quadrant on the left of the primary trochoblasts. The basal cells, the largest of the first quartette, are in contact with the tip cells of the second quartette. A considerable distance therefore exists between the groups of primary trochoblasts, which is filled to a large extent by the basals, the remainder being completed by the accessory trochoblasts.

In *Amphitrite* the cell corresponding to the accessory trochoblast is larger than the basal cell, and this latter, owing to its small size and the relatively large development of the primary trochoblasts, becomes crowded back into the angle between the arms of the rosette series. The basals therefore are not in contact with the tip cells (slight contact in one quadrant of diagram) and the space between the groups of primary trochoblasts is filled with the accessory trochoblast and thus the girdle is complete. But it must be noted that the girdle is also completed by the tip cells of the second quartette, and it is this girdle that becomes functional, the accessory trochoblasts being ultimately pushed above the



functional prototroch. Thus it happens that in arnelids the cells corresponding to the accessory trochoblast never enter the functional prototroch. What their fate may be we do not know. In the posterior quadrant of Amphitrite a product of the accessory cell is very minute, with a darkly staining nucleus, and owing to these characteristics it was used as a "landmark". It was never seen to divide, and to me it has much the appearance of a degenerate cell. In one or two of the remaining quadrants a small cell is figured forming a cell corresponding to the one just mentioned. These also, judging from the figures, have a dense and darkly staining nucleus, but whether they degenerate has not been determined. It would be very interesting to discover if these cells do degenerate and are cast out. If they do it would lend much to the view that these cells were once functional locomotor cells but losing that function they have degenerated as a result.

As regards the cross- In Amphitrite given smaller trochoblasts and a smaller rosette series and a proportionately larger intermediate cell we would have as a

result of the division of the intermediate cells the same conditions that exist in Ishnochiton: in other words there would be an annelid cross similar to the one in Ishnochiton. And conversely: in Ishnochiton if the size of the trochoblasts and rosette series were to be increased and the basal cell were proportionately decreased, the conditions as they exist in Amphitrite would be realized. The arrangement of the homologous cells is practically the same, and the difference in the size of these cells in the annelids and molluscs will explain the presence or absence of the cross.

Turning to gasteropods we find in Hyman's work on *Umbrella* that the first division of the first quartette gives rise to the trochoblasts, and that the second forms the apicals. The resulting stem cells (intermediate cells of annelids: basal cells of gasteropods) are in contact with the tip cells of the second quartette, and consequently a well marked dextrotropic cross is present, similar in all essential particulars to the one in Ishnochiton. The trochoblasts now cleave into two cells in each quadrant but the so-called basal cell of the cross

does not give rise to a cell lying in the arms of the cross but cleaving leiotropically it forms a small cell lying in contact with the primary trochoblasts. In this regard and in the origin, behavior and arrangement of the cells we have the same state of affairs that exists in Ishnochiton. The later stages of the cross in Umbrella are not known save that it is said the basal and tip cells divide, but neither figures nor descriptions give any data for comparison. However, enough has been determined to make it certain that the crosses of Ishnochiton, the annelids and Umbrella in their earlier stages at least are homologous structures.

In Crepidula, as Conklin has shown, a well marked cross exists whose history has been followed much farther than in any other form. Superficially it appears in its earlier stages almost identical with what occurs in Ishnochiton. Closer examination however makes it apparent that although the two crosses have the same position, and same general appearance and for a considerable distance at least much the same history, yet they differ in origin. In both, the first and second divisions of the first

quartette give rise to trochoblasts and apicals respectively, but while the third division is leiotropic in *Ishnochiton*, the *amnelids* and *Umbrella* and therein fulfils the law of alternating cleavages as proposed by Kofoed ('05), it is dextiotropic in *Crepidula*. This is the only well marked case of reversed cleavage in all quadrants that is to be found in *Crepidula* up to the 60 cell stage and it is of very great interest and importance to discover if possible why this reversal occurs.

In *Umbrella* eight trochoblasts form when there are twelve cells in the cross; in *Amphitrite* there are sixteen trochoblasts and probably about twenty-four cells in the cross; in *Clymenella* the same; and as Conklin has stated there are twenty-eight cross cells in *Nereis* when sixteen trochoblasts are formed, while in *Crepidula* there are forty-two cross cells while six or possibly eight velar are present. All of which goes to show that while the development of the cross and consequently the velar field is relatively rapid in *Crepidula*, the velum itself progresses but slowly. This tardy development of the velum is probably due to the lengthening of the pre-larval period owing to the secondary accumulation of consid-



erable yolk. While this delays the division of the trochoblasts and consequently retards the assumption of the free swimming condition, it does not apparently hinder the development of the velar field. However, if *Crepidula* were to form an accessory cell in the same way as is formed in the forms considered above, and at the time when the velum had reached a degree of differentiation represented by two cells in each quadrant the development of the cross would be greatly retarded, and instead of there being forty-two cells in the cross there would be in all probability not more than twelve or sixteen. But since the development of the cross is so precocious or probably better since the differentiation of the velum is so slow I believe the cleavage that normally should form the accessory cell is omitted. Whether the omitted cleavage ever occurs or is in other words postponed is a question that at present cannot be solved.

It is interesting to note that in the anterior arm of *Crepidula plana* arising possibly as a product of the basal cell are two small cells that degenerate and it is believed are ultimately pushed out and thrown away. The thought suggests itself that these two degenerating cells represent products of the accessory cell but there is

nothing to prove this is actually the case.

From the foregoing description it will appear that a cross, such as occurs in *Ishnochiton* and *Umbrella*, is present in the annelids though it does not become apparent, owing, for one reason at least, to the small size of the basal cell; also the cross in these forms is the same as that in *Crepidula*. This latter animal lacks a leiotropic division that is present in the other forms, but after these have passed through that cleavage their position and history, so far as this has been traced, is similar.

Later History- continued. Commencing with the stage as represented in fig. the spindles of the basal cells lie perfectly horizontal and each resulting cleavage forms two cells of equal size. In very many cases I have noticed that the posterior basal cell divided slightly in advance of the others, yet this appears to be of little significance as precocious cleavages of the daughter cells are rather rare at the next division.

The median cells of the cross also appear in a state of division at this time. The cleavage is leiotropic and also results in two cells of about the same

size in each quadrant.

From this time forward cleavages in these cross cells occur with considerable irregularity. I have chosen such a stage as is shown in fig. because it gives an accurate idea of the direction of the cleavages, but as a rule they do not occur so simultaneously. Considering the division of the two basal cells of each quadrant, the cleavages are dextrotropic and leiotropic producing in all but the posterior quadrant relatively smaller products () than the parent cells. In each quadrant they lie on either side of cell of the rosette series and in fig. the position and relative size of these cells in A and C is shown. In the anterior quadrant they are about half the size of these just described but the position is the same. In the posterior quadrant the cells are the same size as the parent cells and this gives rise to a row of four uniformly sized cells that afford a ready means of orientation.

Regarding the median cells, the cleavages are even more irregular and the size of the resulting blastomeres is not constant although the direction of the cleavage is

regular, being dextrotropic as is shown in fig. also in many cases more or less shifting results after the cells are formed.

The study of the cell lineage is impossible from this time on, and it has probably reached a point when it ceases to possess any value in a comparative way. The arms of the cross retain their original position and in the subsequent development form the ectoderm of the head vesicle.

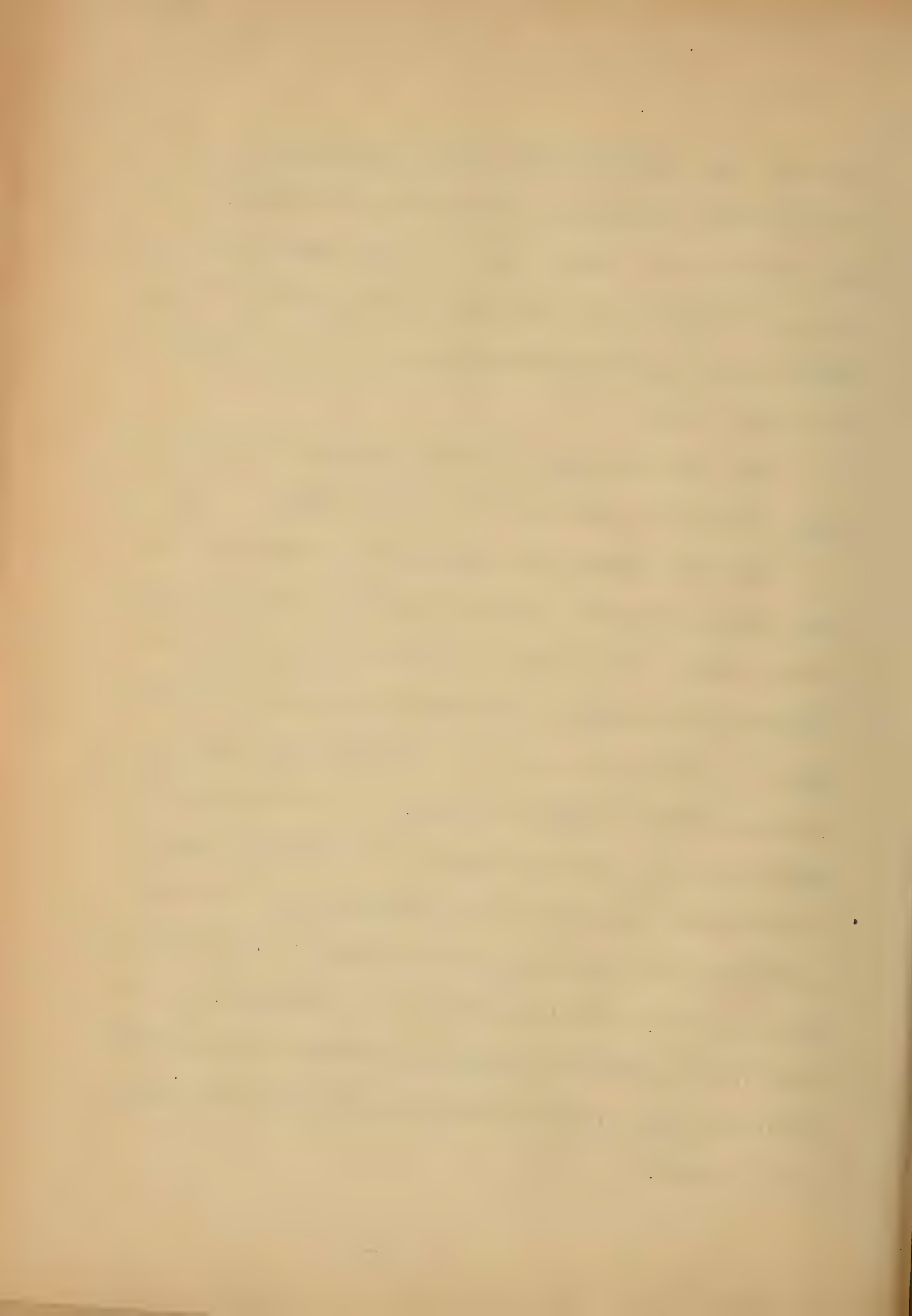
Comparisons.

Before leaving this phase of the development it may not be without interest to compare it with what obtains in *Crepidula*.

Neglecting the division which normally forms the accessory trochoblast, the cross in *Ishnochiton* is precisely similar to that in *Crepidula* up to the time when there are three cells in each arm. The first differences arise in the posterior quadrant which will be considered later. In the remaining quadrants the first division of the basals is identical in each case and the cells formed by the cleavage of the tip and median cells have

much the same appearance to those in Ishnochiton, yet the direction of the spindles are slightly different. But even though this be the case a general resemblance may be traced beyond this point, and in the position of the arms the two forms are closely similar as far as they have been traced.

The posterior arm is rapidly modified from its radial condition as the divisions of the basal and tip cells are teloblastic, ultimately giving rise to a long slender arm one cell wide extending below the level of the velum. The division of the tip cell therefore is different from those in the remaining quadrants and from those in Ishnochiton, and the subsequent cleavages have no resemblances in the two forms. It is interesting to note that after many divisions in the posterior arm of Ishnochiton, which in general resemble those of other quadrants, the cells arrange themselves in rows extending from the rosette series down to the prototroch. They have much the appearance of having been formed by teloblastic divisions but primarily their arrangement is not due to such a cause.



This apparently affords another example of the effects of a bilateral symmetry upon the earlier stages. In forms as widely separated as *Unio* and *Nereis* or *Amphitrite* for example there is an excessive development of the posterior second and fourth quartette cells (first and second somatoblasts) with correlated modifications and the above resemblance between the posterior arms in *Crepidula* and *Ichnochiton* belongs to the same category. In such cases however it by no means follows that the cleavages which produce such characters as the ventral plate and teloblastic arrangement cells are identical. At another time I shall advance some reasons for believing that while precocious segregation tends to produce an early appearance of a bilateral condition it tends to destroy close cell homologies. And in this case of the posterior arms we find that there is a tendency expressed to depart from the radial condition and to assume a teloblastic arrangement although the cleavages producing these cells are not similar.

Rosette series-"Annelid Cross".

The first cells of this group arise in the 24 cell

stage by a dextrotropic division. Each of these products divides by a leiotropic cleavage into two cells (fig.). The central group of four constitute the apical series, and the four peripheral the rosette series.

The next division occurs in the two posterior cells of the rosette series (fig.). In this case there is a hastening of the division in the posterior quadrants, a phenomenon which manifests itself from now on. This division is radial and is generally almost completed before a similar cleavage affects the anterior cells.

Before this latter division is completed spindles appear in the apical cells. In many cases, which I noticed particularly, the first spindle arose in C and was almost immediately followed by D but I cannot lay any stress upon this point for many variations existed. This cleavage is dextrotropic and results in a beautiful cross of cells lying symmetrically about the animal pole (fig.). From this time onward the cleavages vary more and more, lacking regularity except that in almost every case they occur in the posterior quadrants before the anterior.

The next cleavage is in the rosette cells of the posterior quadrants and . In a certain sense this cleavage is bilateral, but with a subsequent similar division in the anterior quadrants it becomes truly radial.

All the cells of the apical series divide in the next stage. The outer ones cleave irregularly in every one of the score of cases that I have studied, but I think that it may be safely said that the division is leiotropic in the majority of cases. Fig. gives a very good idea of the modifications that arise in this division.

The central cells and also divide in a leiotropic division the posterior being shown in fig. .

In the same figure the cells and of the posterior arm of the cross may be seen dividing. The cleavage is perfectly bilateral and is the first one of the rosette series, any others up to this stage being due simply to an acceleration of cleavages which sooner or later appear in the anterior quadrants.

Beyond the stage of 26 cells I have been unable to

continue the cell lineage owing to great irregularities in the time of cleavage, and this difficulty is augmented by the fact that the basal cells of the cross form several small cells that destroy the distinct outlines of the group. However I have been able to follow the cells in a general way to a stage shown in fig. . Here one is reasonably sure of the general position of the series, especially so regarding the anterior arms which remain undivided and no longer exist in contact with the trochoblasts but are cut off from them by the cells of the molluscan cross of adjacent quadrants uniting below them. Also the posterior arms consisting of several cells may be made out with considerable accuracy, and in a general way the entire series may be determined. At a later period the anterior arms of the cross divide but how their cleavages compare with the posterior I am unable to say.

Whether this rosette series forms the cerebral ganglia cannot be decided definitely but there are two or three facts which lead one to believe such may be the case. In the first place the slight indentations over the place where the cerebral ganglia are forming, which

stain intensely black with osmic acid, appear where the rosette series disappeared, and their outer extremities are some distance removed from the velum as was the case with the tip of the arms when the rosette series was last seen. Certainly it can scarcely be questioned that the inner ends of the ganglia in contact with the apical sense organ arise from this annelid cross, which occupies the exact centre of the velar field. Also the rosette series shows throughout its development a remarkable independence. Its cell cleavages in no way appear closely correlated with those of the arms of the molluscan cross, and as far as it can be traced it exhibits a quadriradial symmetry in its outline. And it is certainly a significant fact that appearing exactly as do the cerebral ganglia are two smaller areas posterior to the sense organ and connected with it. I have examined hundreds of embryos at this stage, and this character is always present and I feel positive that small masses of cells proliferate in these areas, which uniting with the inner ends of the cerebral ganglia lie beneath and in contact with the apical sense organ. In rare cases surface views show

them extending to a greater distance, and in three embryos I have found the two posterior tracts to extend to as great a distance as the cerebral ganglia. They were slightly depressed but lacked the perfect sharpness of outline characteristic of the cerebral ganglia, but otherwise exhibited much the same appearance as these latter structures.

The thought suggests itself that the rosette series with its independence of development and radial form is to be considered as the fundament of a quadriradial nervous system which in the primitive ancestor developed in all quadrants but which has partially degenerated owing to the change from a radial to a bilateral form. Nervous systems similar to this are found in the *Polygordius* trochophore and the Ctenophores, but the manner in which these develop is unknown, yet from the behavior of the rosette series and the development of the nervous system I am led very strongly to the belief that the primitive form of nervous system was, as was the ancestor, radial.

Second Quartette.

The second quartette takes a lesser part in the de-

velorment than does the first or third, yet it is not second as regards the diversity of its development. From its cells arise a considerable portion of the stomodaeum, a part of the shell, probably the pedal cords, certainly a part of the foot and to a very slight extent a portion of the body epithelium on each side of the mouth. It arises early in the development, in the sixteen cell stage, and may be followed for a long period.

The cells of this quartette arise by a leiotropic division of the macromeres at the same time that the formation of the trochoblasts occurs, and abutting against the latter they occupy furrows between the macromeres (fig.

The second cleavage occurs simultaneously with the division of the trochoblasts and the resulting group occupies its original position. This division was dextro-tropic resulting in the formation of a smaller superior cell and a larger superior one (fig.).

At the next cleavage both cells are affected, the upper product first showing signs of division in which are formed two cells, the larger upper left hand one being the tip cell of the molluscan cross, destined, except

in the posterior quadrant, to furnish two cells in each quadrant functioning in the prototroch; the remaining relatively slender cell enters into the "supporting layer" of the prototroch and is probably to be considered in the nature of a post trochal cell.

From this time on the development of this quartette in the posterior quadrant is along entirely different lines from the remaining and for this reason it will be omitted in the present account and later considered under the history of the first somatoblast.

As mentioned, the following division does not commence until the previous one has advanced beyond its initial stages. It results in the formation of a cell almost identical with the one formed in the above division and not only is it similar in appearance but their destiny is the same, both being post trochal cells.

Considering the cells at this time we find each group nearly bilaterally symmetrical with reference to a principal axis meridian passing through it. The blastomeres are grouped in the form of a Y the stem being $2a^{2,2}$ etc., the limbs $2a^{1,2}$ and $2a^{2,1}$ etc., while supported in the

angle between these latter cells is the tip cell $2a'''$ etc. For the only time in their history the tip cells now divide. The cleavage is dextrotropic and produces two cells which enter the functional velum, and following Mead are termed the secondary Trochoblasts.

The spindles introducing the next cleavage are perfectly meridional and the completed division results in two products in three quadrants, the lower smaller ones being situated between the parent cells and the fourth quartette. These cells ($2a^{2.2.2}$ etc) enter into the formation of the stomodaeum and are the secondary stomatoblasts.

The cleavage following this occurs simultaneously in $2a^{1.2}$ and $2a^{2.1}$ etc., being leiotropic and dextrotropic respectively. I believe that these cells do not divide again, certainly not up to the point where they form an almost continuous row about the embryo.

As a usual thing before the above cleavage is complete another occurs in $2a^{2.2.1}$ etc. producing two cells of almost equal size lying one above the other.

The next division occurs in the anterior secondary

stomatoblast . The spindle occupies the plane represented by the arrow in fig. . The innermost cell is generally about two thirds the size of the parent cell and lies directly upon the fourth quartette cell which is usually in a process of division at this time.

The cells $2a^{2,2,1,1}$ etc. now divide into a right and left half of equal size.

The division of the right and left secondary stomatoblasts occurs at the same time as the above and the characteristics of the cleavage are best described by the drawing (fig.). The approach of the ectoderm cells towards the vegetative pole causes these cells to be crowded into the embryo. As a usual thing the tertiary stomatoblasts encroach upon them to such an extent that at the time of their division very little of their surface is exposed. It is to be noticed that this cleavage is bilateral.

From this point I have been unable to trace the cell lineage of the entire group. But at a later period it appears that some divisions have occurred the origin of which may be surmised. In fig. it appears that

have divided transversely and have produced four

cells; that the lower cell has by transverse division given rise to a single row of cells passing into the blastopore margin. In some cases there appear to be three such cells but I cannot be positive on this point.

At a time shown in fig. the lower hemisphere is somewhat triangular owing, as will appear later in the account, to a tendency on the part of the third quartette cells to press in upon the archenteron. At such a time the greater portion of the second quartette stands out boldly forming the angles of the triangle. Later however when the embryo resumes a circular form the second quartette sinks slightly below the general surface, forming grooves which are of the greatest value in late stages, as indicating the region of these groups of cells. The anterior groove is wider and more open than the right and left, and as the mouth shifts along this path, I believe that many, and possibly the majority, of the second quartette cells disappear into the stomodaeum. There can scarcely be any doubt but that there is an invagination of at least a part of the anterior second quartette up to a time when the blastopore has shifted 45° and I believe

it continues until the shifting is complete.

In the right and left groups of this quartette the invagination is small as the mouth moves into its first position. The cells however retain their connection with the blastopore and prototroch and as the shifting occurs they become curved until at the time when the mouth has about reached its permanent position they are nearly perfect semicircles bordering the third quartette cells lying on each side of the mouth.

We are now in a position to consider the fate of these three groups of cells. As I have said I believe that little doubt exists that most if not all the cells in the anterior quadrant become swallowed up and enter the stomodaeum. The right and left groups are relatively slender, forming narrow curved strips lateral to the mouth and probably contribute to the formation of the general surface of the body.

Comparisons.

Conklin in his study of *Crepidula* has made exhaustive comparisons of various molluscs relating to this quartette, and finds very close correspondence both as re-

gards direction of cleavage and position of cells. For this reason therefore I shall simply compare *Crepidula* and *Ishnochiton*.

The divisions up to the formation of four cells in each quadrant are exactly as in *Ishnochiton*. But in the next cleavage in *Crepidula* $2a^{12}$ divides in a "slightly leiotropic" direction, and therein conflicts with the regular alternating cleavages and consequently with *Ishnochiton*. However, very shortly after their formation these blastomeres shift so that they have exactly the same position in the two forms.

The next two divisions (of $2a^{11}$ and $2a^{14}$) are also similar, but after this point comparisons cannot be drawn since at the next divisions in *Crepidula* the cells corresponding to the post trochal cells divide a second time, cleavages which certainly do not occur in *Ishnochiton* previous to the three or four hundred cell stage.

Therefore up to the time when there are seven cells in each quadrant the same features of development obtain, save in the one case where the cleavage was slightly lei-

tropic. However, it must not be understood that the second quartette in Ishnochiton when these cleavages are completed is similar to that of Crepidula, for the cell $2a^{2,2}$ which in Crepidula has never been seen to divide, in Ishnochiton divided repeatedly.

This brings out an interesting point in Crepidula, that while the upper cells of this series are similarly arranged to those in chiton the division which produces the stomatoblast is delayed. This is probably correlated with the late formation of the stomodaeum.

Later stages in the cell lineage were not determined owing to a lack of landmarks, which latter fact rendered it impossible to accurately differentiate the second from the neighboring third quartettes. However, in a general way these groups have been followed, and some features in their development are strikingly like those in Ishnochiton. For example, during the early stages of gastrulation, the second quartette cells extend from the arms of the cross to the angles of the quadrangular blastopore, while the third quartette cells extending the same distance alternate with them around the embryo. Com-

paring this with figs. in Ishnochiton, we have exactly the same conditions. Also it is believed that in all but the posterior quadrant, some cells from this quartette contribute to the formation of the velum: in other words they form secondary trochoblasts as in Ishnochiton.

A growing point forms in the second quartette of the posterior quadrant which also occurs in chiton.

Beyond this point comparisons cannot be made, but as far as it has been traced the development in the two forms is very similar and there is every reason to believe that if it were possible to carry on the comparison into later stages these resemblances would not cease.

Annelids. Among the annelids this quartette appears to be subject to considerable variation as regards the size of cells, and the accounts of their various fates differ considerably. In Nereis, according to Wilson, $a^{2,4}$, $b^{2,4}$ and $c^{2,4}$ are destined to enter the stomodaeum, which observation in general meets with general approval: but regarding the destiny of the parent cells $a^{2,4}$ etc. differences of opinion arise. According to Wilson the descendants of these cells divide more or less vertically, giving rise to "a series of large polygonal cells lying be-

low the "prototroch" which he terms post trochal cells. None of these are said to enter the prototroch as secondary trochoblasts, but from the anterior group with the accompanying descendants of a^3 and b^2 "arises the ectoblast surrounding the stomodaeum and forming the superficial part of the body-wall of the antero-lateral region"; and regarding the fate of these cells in the right and left quadrants he says "the postero-lateral region on each side (between the margin of the ventral plate and the prototroch) is occupied by the descendants of the post trochal cells (offspring of $a^{2.1}$ on the left side, and of $c^{2.1}$ on the right) and of c^3 and d^3 ."

These various statements have been called in question by both Lillie and Mead, and the objections are strengthened by the history of these cells in *Ishnochiton*. Mead finds in both *Amphitrite* and *Clymenella* that the second quartette in the three quadrants furnish secondary trochoblasts and the same thing occurs in *Ishnochiton* and probably in *Crepidula*. Also Lillie and Mead criticise Wilson's statement that $a^{2.1}$ and $c^{2.1}$ aid in the formation of the latero-dorsal region. In *Unio* the second stomato-

blast occupies this place and in Amphitrite since the descendants of 2d form almost the entire trunk such a fate for these cells is out of the question. In Ishnochiton this region is formed by the products of d^2 and also by the dorsal borders of 3c and 3d.

There is thus a wide difference of opinion regarding the fate of these cells, but since there is such an essential agreement in this regard between the development of Unio, Amphitrite and Ishnochiton it seems probable that Nereis will be found to offer no exception when restudied; and it also seems probable that in the majority of annelids and molluscs the second quartette will furnish secondary trochoblasts and will extend from the prototroch to the blastopore even though the development of the first somatoblast introduces a great distortion in the original radial symmetry.

First Somatoblast.

Attention has already been directed to the fact that in the early stages of development no appreciable difference in the size of the second quartette cells exists, and that the first cleavage is the same throughout; the same thing is true of the next division which occurs in the upper of the two blastomeres producing the tip

cells¹¹ etc. and post trochal cells $2a'^{12}$. At the next division, however, close resemblances in the lower cell cease for in the division of $2d^2$ stomatoblast is formed while the division of $2a^2$ etc. produces post trochal cells. The significance of this cleavage has been previously considered (cf. page).

The next stomatoblast cleavage occurs in the tip cell ($2d^1$) and is precocious when compared with this division in the other quadrants, but otherwise it is exactly similar to them.

The cell $2d^{11}$ now divides; the spindle is leiotropic and produces a cell which occupies the same position that $2a^{11}$ etc. does in the other quadrants. It appears that this cleavage with reference to the one producing the stomatoblast, $2d^{12}$, is completely reversed; and it is a curious fact that both of the cleavages producing these two cells are leiotropic.

The stomatoblast $2d^{12}$ now divides transversely into two cells of equal size. This division consequently does not correspond in direction to that of the other secondary stomatoblasts.

The next cleavages occur in $2d^{11.1}$ and $2d^{11.2}$ which

which are the products formed by the division of the tip cell; the cleavages are dextiotropic and leiotropic, respectively (fig.). In the other quadrants the cells $2a^{11}$ and $2a^{12}$ etc. are secondary trochoblasts and never divide, but in the posterior quadrant they have become secondarily modified and divide several times.

A spindle now soon appears in $2d^{21}$ dividing the cell leiotropically into two equal moieties.

There are now nine cells in the ventral plate, and the number becomes rapidly increased, but I have been unable to determine the order in which they occur. However, I have seen the division of $2a^{11,12}$ and $2a^{13,14}$ (leiotropic); of $2d^{13}$ (dextiotropic) and of $2d^{21,22}$ (horizontal).

The cells at this time, for a considerable period of their development, are relatively smaller than the neighboring third quartette products, and they are by no means so regularly arranged; consequently it is not a difficult task to determine the outlines of the somatoblast up to the stage shown in fig. . While this group as a whole increases rapidly in size it will be noticed that this applies principally to that part between the prototroch and the lower border of the growth zone.

The portion below this consists of a row of cells which extend to the blastopore, and for a considerable length of time (during the first stages of invagination) they occupy the bottom of a groove whose sides are formed by the larger third quartette cells. Later, as this latter quartette becomes composed of cells of smaller size the groove disappears and this part of the somatoblast becomes ill-defined. The upper portion on the other hand remains distinct for a longer period and gradually extends itself over a larger area on the dorsal region. When it ceases to be distinct as shown in fig. it extends over nearly the area occupied by the future shell when this latter structure first appears, and I think there is no doubt but that at such a time the greater portion of this organ forms from the somatoblast.

What part the somatoblast plays in the development of the ventral surface it is impossible to state, but no doubt it forms a much smaller portion than the third quartette. It is probable that in such forms as *Amphitrite*, where the entire trunk forms from 2d, the ventral nerve cord arises from this cell. It may do so in *Ishnochiton*,

but since this part of the nervous system is probably a secondary acquisition it does not necessarily follow that it arises from the same cell in different groups of animals. However, there is no doubt but that the somatoblast in *Ishnochiton* forms most of the shell; a small portion of the posterior part of the body, including mantle and mantle furrow, and a portion of the foot.

Comparisons.

In *Ishnochiton* it is readily seen that the first somatoblast arises from a cell which in origin, size, and position is similar to the other second quartette cells, and that in all probability its ^{relatively} excessive development has been secondarily acquired owing to the shifting of the mouth and formation of a ventral surface. Consequently it is reasonable to suppose that in the original radial ancestor the second quartette developed in the same manner in each quadrant.

Among molluscs, *Crepidula* shows more strikingly than *Ishnochiton* the radial symmetry of the second quartette. As far as the cell lineage has been followed (up to a time when eleven cells in each quadrant have been

formed), the changes have been similar in all quadrants. Beyond this point differences between the posterior group and the three remaining arise, but the manner in which this is brought about is not known.

In annelids whose cleavage belongs to the "equal type" (*Lepidonotus*, *Podarke*) there is at first no distinction in size among the second quartette cells, and Treadwell has shown for *Podarke* that the first divergence for development occurs in the posterior group, upon the formation of four cells in each quadrant. At this time the cleavages are similar throughout, but there is a noticeable difference in the size of the first somatoblast products as compared with corresponding cells of the remaining quadrants. Among other annelids, such as *Nereis*, *Amphitrite*, and *Arenicola*, the effect of the bilateral form of the larva is more pronounced, manifesting itself at the first cleavage. The same is also true of the lamellibranch, *Unio*.

The somatoblast in these cases is characterised by a relatively large size, and the typical radial symmetry of this quartette is proportionately disturbed. This divergence becomes more marked as the cells commence to de-

velop, when it is very difficult, if not impossible, to homologize the cleavages of the first somatoblast and the other quadrants. A similar difficulty is also experienced when an attempt is made to homologize first somatoblast cleavages in embryos of two animals in different classes, and it is not much lessened even in a comparison of embryos in the same class.

The difficulty is not so great if one attempts to homologize the remaining second quartette cells and it appears probable as mentioned above that in the original ancestor this quartette underwent a similar development in all quadrants. A secondary shifting of the axes has produced secondary changes in 2d, but it by no means necessarily follows that such secondary effects should manifest themselves in exactly the same fashion in different classes or even genera; indeed it would be strange if they did.

Finally, Mead has made the interesting discovery in *Amphitrite*, that with the exception of small areas in the immediate vicinity of the mouth the entire trunk forms from 2d. In *chiton* the trunk in its early stages is al-

most as extensive as in this annelid but at least one half of it is formed from the third quartette, which in annelids evidently remains of small size.

Third Quartette.

The history of the third quartette is the simplest and least varied in the development of the ectoblast. At its first division it produces the third quartette stomatoblasts, whose fate is considered on page . To the remaining products attention will here be paid. Their first division is meridional, the spindle being horizontal, and as a result two cells of the same size are produced in each quadrant. In this and the succeeding cleavages of this quartette the posterior quadrants are the first to show signs of division. However, their acceleration is at first not of much consequence as a glance at fig. will show. The next division is at right angles to the first, and affects all the cells produced by the above division, etc. as in the preceding case the result is an equal cleavage in each cell, making a quadrangular group of four cells in each quadrant. The acceleration of cleavages is here more marked (fig.).

The next cleavage occurs in the upper cells in each group () and is later followed by the lower products (). The spindles of both these cleavages are horizontal, and are at right angles to the preceding. In this case each cell cleaves into equal products, and the division is usually completed in the posterior cells before it has advanced to the stage of the metaphase in the anterior quadrants.

The time difference between the anterior and posterior quadrants soon becomes very marked and an irregularity in the direction of the anterior cleavages sooner or later creeps in so that they become a confused irregular group of cells occupying the space right and left of the mid line between the second quartette blastopore and prototroch. The cleavages do not cease but the cell growth is apparently small for while the cells become more numerous they do not expose a greater surface as a result. This continues as far as the group can be followed, which represents a stage when the blastopore has shifted nearly to the prototroch, and it appears probable that it never occupies a much greater area than it did early in its

history.

In the first quartettes however matters are different. In the upper dorsal portions of each of the two groups the cells continue to possess a great regularity, exhibiting the appearance of stones in a wall; but in those portions anterior to the growth zone and forming the ventral surface of the embryo the cell divisions early become irregular.

The growth zone which becomes established before the blastopore has shifted to any great extent appears to arise in the neighborhood of the prototroch and gradually work downward. It has already been noticed that the upper cells of the third quartette divide before the lower (fig.) and fig. shows a series of cleavages in the row next to the top while in fig. the most rapid cell divisions have occurred in the fifth row. At a slight distance below this point the growth zone becomes located permanently, which brings the area of the most active multiplication of cells immediately posterior to the shell, a position which it occupies in gastropods and its situation corresponds closely to the same region

in annelids.

The increase in area of these posterior third quartette tracts is not, except to a slight extent, in arcs of circles parallel to the prototroch, though there is some lateral displacement caused by the growth of the first somatoblast on the dorsal side. The main growth is at right angles to the velum and the distance between the posterior border of the blastopore and the prototroch becomes continually increased. Also through invagination the distance between the blastopore and velum on the anterior side becomes lessened and in proportion as the former shifts toward its future position these posterior third quartette areas become extended beyond the first position of the blastopore, up along the original side until they come in contact with the prototroch, thus forming most of the ventral side of the future embryo.

Probably about half of the trunk of the embryo is formed of these posterior third quartette cells. Each group contributes to the lateral borders of the dorsal surface, the products of the first somatoblast forming the triangular mid-dorsal region: the posterior lateral

regions arise wholly from these cells, as does also most of the ventral surface. Diagrams will show more clearly the position of this quartette.

As regards the third quartette cells of the anterior quadrants, little remains to be said. In their first cleavages a very marked regularity existed but this gradually disappears and an irregular group of cells results. Cell divisions continue but the increase of superficial extent in the groups is very small, apparently little more than in the earlier stages. I have studied the matter quite closely and am convinced that they always remain thus relatively small and are of comparatively little importance in the history of the embryo. They have been traced up to a point where the blastopore has almost reached its permanent position and at such a time they form two areas on each side of the mouth in juxtaposition with the prototroch as shown in fig. . It is possible that they include the antero-lateral borders of the foot when it is formed. In any event they probably function as simple ectodermal cells, many if not all of which lie between the first and future shell and consequently in

the metamorphosis they must in great part lie in the mantle furrow.

Comparisons.

In all other forms whose development is accurately known the third quartette is very much smaller than in *Ishnochiton* and the part it performs in the development is not so striking as in *Ishnochiton*. Whether or not it is as important or not cannot be answered at the present time since no one has described the cleavages beyond the earlier stages.

In *Umbrella* and *Crepidula* among molluscs the first division of this quartette is nearly radial, but a shifting occurs which throws the cells into the same position as in *Ishnochiton*. The next division affects the upper cells, cleaving them bilaterally while a subsequent cleavage divides the lower cell (stomatoblast) into two equal halves (this is not described in the two posterior quadrants in *Umbrella*). These last two divisions are very similar in *Crepidula* and *Umbrella*, and correspond closely to the divisions of this quartette in *Ishnochiton*. Beyond this stage no cleavages in the anterior quadrants

are figured in Umbrella, and in the posterior modifications arise owing possibly to the fact that some of these cells become excretory.

In *Crepidula* the outer cells, corresponding to the posterior third quartette stomatoblasts, and , in *Ishnochiton*, divide into upper and lower products. This cleavage occurs in this direction in chiton but much later in the development. Also there is another relatively accelerated cleavage in *Crepidula* in the division of $3c^{1,2}$ and $3d^{1,2}$. The corresponding cleavage in the anterior quadrants occurs much later, and at the same time the upper cells $3a^{1,4}$ and $3b^{1,4}$ divide. Hence in the anterior quadrants the two third quartette groups (Conklin's fig. 47) at this time bear a striking resemblance to our fig. There are two lower cells in each group corresponding to the stomatoblasts: two median cells ; and two upper that have the same origin and position in the two forms.

Regarding the later history of these cells nothing is accurately known.

Invagination.

After the formation of the third quartette, the

macromeres lying at the vegetative pole occupy the furrow between the members of this quartette. A segmentation cavity of considerable size is now present but they project into it but little. With the formation of the stomatoblasts of the third quartette, however, the ectoderm extends nearer the vegetative pole and in proportion as the macromeres are encroached upon they pass into the interior. This is manifested by a change in the leiotropic position of these cells which become strictly radial; by a more compressed appearance as indicated by a polar furrow of greater length and a loss of roundness of cell contour in the exposed portions; and also by the form of the cells which are clearly pearshaped with the larger end bordering the segmentation cavity. This is the first step in the invagination process and may be said to commence in the initial stages of the 36 cell embryo.

The cell from which the mesoblast arises is generally slightly the largest of the macromeres. Not infrequently this is not manifested by the external surface, but in such cases it is found to have migrated to some degree into the interior. After the mesoblast is formed

this inward movement generally becomes quite pronounced, and by it less and less of the external surface remains exposed. The process appears as a consequence of the actual inward movement of the mesoblast cell, accompanied by the overgrowth of the mesoblast by the posterior members of the third quartette. A comparison of the figures of the early and late stages of invagination will show that as the mesoblast passes in, the stomatoblasts at first widely separated gradually cover the former and in so doing approach each other along the antero-posterior axis.

In the cell stage the remaining members of the fourth quartette form. The position of the spindles is radial and the division likewise.

Somewhat later than the preceding division spindles appear with bilateral arrangement in the third quartette stomatoblasts. The resulting cleavage divided the anterior cells into equal moieties, but the posterior cells each bud off a small cell anteriorly that is crowded in between the parent cell and the neighboring fourth quartette.

Movements among the cells on the vegetative pole, such as have been mentioned, are usually observed previous to the stage last described, but they may be said to be premonitory since no very marked invagination has occurred, the only effect being a slight decrease in the external surfaces of the macromeres and the mesoblast cell accompanied by a flattening of the vegetative pole. With this last cleavage of the stomatoblasts invagination may be said to fairly commence, but in order to understand its processes together with the shifting of the blastopore it will be necessary to consider in some detail the relations of the cells on the vegetative pole.

In a stage previous to the division of the third quartette stomatoblasts as shown in fig. the four macromeres lie at the vegetative pole. The cell, D, is as has been maintained usually very small comparatively, and as a result it plays a very inconspicuous role during invagination. The three remaining macromeres on the other hand are of considerable size extending within the embryo to a point perhaps level with the prototroch.

In the formation of the fourth quartette the macromeres in quadrants A, B and C divide externally into almost equal products, but following the cleavage furrow into the interior of the egg it is found that the fourth quartette cells become thinner and wedge like and that the macromeres are consequently thickened and pear shaped. A general idea of the shape of these cells may be gained from fig. . The inner ends of these cells are at about the same level.

The mesoblast is a larger cell than any of the others mentioned, the largest in fact on the vegetative pole of the egg, and more than this needs no mention more than to state that the inner end of this cell is about on the same level as the inner ends of the macromeres of the other quadrants.

The stomatoblast cells of the second quartette () are at this period almost wholly without the region of the blastopore. They will be more fully discussed later on when they come to take part in the formation of the stomodaeum.

The third quartette stomatoblasts are much more im-

portant cells. In size they are superficially larger than any of the cells on the vegetative pole except the mesoblast, and superficially they appear of about the same size, but a careful study of stained preparations and wax reconstructions at this stage make it clearly apparent that in reality there is a considerable disparity in the masses of these cells. The two anterior ones, roughly speaking, are ellipsoids, extending therefore but a short distance into the egg, being wedged in position between the cells of the third and fourth quartette and the macromeres. For example, not infrequently $3b^{ab}$ and $3b^{ab}$ are in contact with $4b, B, 4a$ and A , a little distance within the embryo, and the stomatoblast thus occupies a trough between the cells. Not so the posterior cells; lying closely adpressed against the mesoblast they extend inward often nearly to the same distance and the third quartette therefore is always separated from the mesoblast. This latter with its two companion stomatoblasts constitutes a system that is but slowly affected by invagination, upon which, probably, depends to a large degree the shifting of the blastopore.

Occasionally the first cells to invaginate are the fourth quartette cells in quadrants A. B and C. which in some cases disappear wholly from view before any other cell exhibits a similar movement. In other rare cases where the cells are of normal size and arrangement the same thing may be said of the mesoblast, while in cases where D or 2d are larger than usual the mesoblast usually passes into the embryo at an early stage. But the normal invagination commences shortly after the division of the third quartette stomatoblasts. Almost from the moment of its formation the mesoblast has been slowly moving inward, but it is almost imperceptible and therein differs from the general normal invagination movement which affects first all the three macromeres A. B and C. These when compared with the mesoblast glide with considerable rapidity past the fourth quartette cells, with which they are in contact, and push into the segmentation cavity. In the first stages of this movement the fourth quartette is not affected but as the macromeres advance the former become gradually depressed and slowly pass with them into the embryo. Shortly after the movements initiated by the

macromeres commence, Macromere D commences to move in the wake of the other macromeres, especially if it be of considerable size: if it be small it may remain for some time attached in its original position to the mesoblast. As the macromeres disappear from view it is seen that they gradually fill the segmentation cavity, and what is now important they press in above the mesoblast. Since their movement is the more rapid there must result a tendency to check the inward movement of the mesoblast. At all events that is what happens. From this time forward the movements of the mesoblastic products become slower and slower and ultimately cease altogether, and therefore the invagination in the anterior quadrants A and B is much more rapid than that in C and D. Considering A and B first we find after the macromeres in their inward movement have been followed a short distance by the fourth quartette cells, that a similar movement occurs in the third quartette stomatoblasts. These are two in number in each quadrant and when viewed from the vegetative pole are at a higher level than the fourth quartette cells and are also above the narrow spaces that separate these lat-

ter cells from each other. These spaces as the cells pass inward gradually enlarge and into them the stomatoblasts gradually work their way. Large as they are in fig. this process would probably be accomplished with difficulty but at this point each of the two cells in each quadrant divides into four cells and these move rapidly into the gap. The anterior half of the archenteron therefore consists of a roof of these macromeres with side walls composed of fourth quartette cells in the anterior posterior line and at positions 90° distant in the right and left quadrants, while between them lie the products of the third quartette stomatoblasts.

Now to the formation of the posterior wall, as I have said, the stomatoblasts of the third quartette in quadrants C and D in their first division give rise to small cells that are relatively unimportant, while the remaining cells are much larger and lying by the side of the mesoblast accompany it deep in to the egg. As the mesoblast sinks in the movement of the stomatoblasts is in an opposite direction, by which they come to lie almost wholly on the surface of the embryo as in fig. , at the same time approaching each other along the median

ventral line. They now divide forming five cells in each quadrant, and continuing their movement inward become almost lost to view. Sections show that at about this point their invagination ceases and for a considerable period they may be seen on the posterior lip of the blastopore in connection with the lower surface of the mesoblast.

Sections or entire embryos at this stage will show that even though the direct inward movement of the mesoblast is checked there is a shifting of its cells by which they come to lie with their anterior surfaces in line with the inner surface of the archenteron while the posterior surfaces extend far backward and also downward into the ectoderm of the posterior half of the embryo. A glance at diagram may make this point clear. Were all the fourth quartette cells alike (4 D mesoblast) would be similar to 4a on the right of the diagram. In that case in the invagination the macromeres would slip past the fourth quartette cells as in the right half of the diagram. But 4d has become modified; it has grown larger than its homologues in the other quadrants and, of the

greatest importance perhaps, it is globular in form. Owing to this latter peculiarity, the macromeres press in above this cell and it becomes pushed outward and downward. Thus a distinct bulging occurs on the lower posterior side of the embryo and the ectoderm in this region becomes comparatively thin, much thinner than at any point on the anterior side of the embryo, and this character is apparently correlated with more rapid cell division in this region by which in a relatively short time the ectoderm cells of the posterior side of the egg double those of the anterior.

Thus in the embryo two agencies are active in producing a shifting of the blastopore; first, on the posterior side there is little or no invagination after the earlier stages but a rapid increase of ectodermal cells by which the area between the prototroch and blastopore is constantly increasing, second, on the anterior side of the egg cell divisions are of less frequent occurrence and an invagination decreases the ectoblastic area lying between the blastopore and the prototroch thus bringing the blastopore ultimately next to the prototroch.

The final changes after the invagination of the third quartette of stomatoblasts are relatively much longer than were the movements up to this period, and it appears that the first movements of invagination up to the point where the anterior third quartette stomatoblasts disappear from view, are relatively so rapid because the cells are crowding into the segmentation cavity. When the cells have invaginated, as long as space is left, a more or less stable equilibrium is reached. After this period for every decrease of space between the blastopore and prototroch there must be a proportionate increase in the posterior surface of the embryo, that is supposing, as appears to be the case here, that there is no farther invagination in the posterior part of the blastopore.

In order that it might not complicate the account of the invagination to a greater degree I have left out the part the second quartette plays in the process. This is the more permissible since, for some time, this quartette does not actively participate in the general movement, though influenced by it in the earlier stages. For example in the cell stage the cells in question are

situated without the circle of cells that will invaginate and in the stage shown in fig. they have advanced but a short distance into that territory. Later when the macromeres are considerably depressed beneath the general surface and the fourth quartette has commenced to take part in the process the second quartette stomatoblasts

are seen to lie within the blastopore area, having advanced as the fourth quartette cells decreased in external surface. They also are below the general level, being wedged in between the stomatoblasts of the third quartette. The movements of these cells are essentially the same in all quadrants but differences in their size cause some changes which modify the external appearance of the embryo. For example the cell in the anterior quadrant is always the largest; the one in the posterior normally the smallest while the cells of the right and left quadrants are intermediate in size.

In such a stage as is represented by fig. the channel between the two sets of anterior third quartette stomatoblasts is shallow and never in the history of the embryo does a deep groove extend from the blastopore to the prototroch. In the right and left quadrants where

the stomatoblasts are smaller they come to lie at the bottom of comparatively deep furrows, between the groups of third quartette stomatoblasts, and these grooves continue to deepen and to extend to other members of the second quartette as the larger cells of the third quartette approach nearer to the blastopore (fig.). For some time I thought this might be correlated with a formation of larval mesoblast but it appears that this is incorrect, for the cells with the exception of the stomatoblasts and possibly the adjacent cell of the second quartette, again come to the general level when the products of the second and third quartettes are more nearly equal in size. In the posterior quadrant the stomatoblast cell divides into two cells that are small and also occupy a deep furrow that extends in some cases half way on to the prototroch. No cells lie in this furrow except those of the second quartette which are of small size when compared with the adjacent members of the third quartette. In each case these grooves appear to have no deeper significance than that they are a result of a compression of small cells between much larger ones, in which the results

are more strongly marked than they would be for example on the anterior hemisphere, owing to a greater pressure than normal produced by the cells of the lower pole crowding into the space left by the invaginated cells.

Comparisons.

The earlier stages in the gastrulation of *Chiton marmoratus* and *C. squamosus* have been studied by Metcalf without however determining accurately the cell lineage. This has led I believe to some errors in the interpretation of certain points which may possibly be corrected in the light of the development of *Ishnochiton* as the relation of the cells in the two forms is almost identical.

In the first place it is an interesting fact that the macromeres and the cells corresponding to the fourth quartette appear to have been formed by spiral cleavages; that they are also remarkably small cells and having above all no difference in their size in the various quadrants. *Chiton* is a more primitive genus in external respects at least than *Ishnochiton* and it would be of interest and importance to determine if this uniformity of cell mass in the macromeres and fourth quartette

represents a primitive condition where the mesoblast is less differentiated from the endoderm than in *Ishnochiton*.

The macromeres are first affected in the invagination. As they press into the interior the cells of the third quartette divide, forming cells homologous with the third quartette stomatoblast. All are relatively enormous cells of the same size in each quadrant, and possibly in the formation of the archenteron compensate for the small size of the endoderm cells. Metcalf speaks of these cells (stomatoblasts) as endoderm. It is true they enter into the formation of the archenteron, but there is the strongest reason to believe that they form part of the stomodæum and are consequently ectodermal cells. As these latter press in toward the vegetative pole the macromeres pass completely within the embryo. The third quartette now divides a second time, forming two cells that are the homologues of in *Ishnochiton*.

In each quadrant between the stomatoblasts is a small cell marked "?" whose origin was not accurately determined by Metcalf though it was thought that they correspond to the fourth quartette. This is probably cor-

rect and the cells therefore correspond in fig. to and . If the view be sound that these cells are the homologues of the fourth quartette then one of them must be the mesoblast. That this interpretation is correct is shown, I believe, by one of Metcalf's figures (diagram) Invagination here has progressed farther than in fig. of the same diagram yet the cells maintain essentially the same positions. One of the cells, a fourth quartette product has divided. Comparing this with fig. practically the same conditions prevail. The only difference is that the third quartette stomatoblasts have not divided. In Ishnochiton also one of the fourth quartette cells (mesoblast) has formed two cells. The resemblance seems too striking to be a mere coincidence and the cells in their origin and history up to this time are the same, neglecting minor differences, and I believe therefore that in diagram Metcalf has figured the mesoblast.

Metcalf also mentions a furrow in the mid-ventral line extending anteriorly from the blastopore out toward the prototroch, and terminating in an enlargement (diagram). As invagination proceeds and the blastopore narrows this furrow disappears. It is said however that

this same furrow reappears when the blastopore commences to shift to its permanent position.

In fig. is found just such a furrow as Metcalf describes. It extends from the blastopore out towards the velum and terminates in an enlargement. Also at its commencement it includes the two products of the fourth quartette cell (mesoblast).

Since in Chiton these two cells lying in the groove are in all probability mesoblast, it follows that the furrow does not extend anteriorly but posteriorly, and the furrow between the blastopore and the prototroch appearing when the former commences to shift is not the same one but another which lies on the anterior side of the embryo as is found also in Ishnochiton. At such a stage landmarks are not easily discovered and therefore it might readily be supposed that these two furrows, the posterior and anterior occur in the same band of cells, but if a more careful study of the cell lineage is possible I feel confident that Chiton and Ishnochiton will exhibit the same phenomena.

Kowalevski () in a brief account describes some

highly unique changes in the development of the blastopore of Chiton Polii. I quote Halfour's¹ succinct abstract at this point. The embryo has probably reached a stage similar to that in fig. and of the following changes he says: "In the succeeding developmental period the blastopore which has so far had the form of a circular pore at the posterior extremity of the body, undergoes a series of very remarkable changes. In conjunction with the gradual elongation of the larva it travels to the ventral side and is prolonged forwards to the velum as a groove. The middle part of the groove is next converted into a tube which opens externally in front and posteriorly communicates with the archenteron. The walls of this tube subsequently fuse together, obliterating the lumen, and necessarily causing at the same time the closure of the blastopore. The tube itself becomes thereby converted into a plate of cells on the ventral surface between the epiblast and hypoblast."

The nervous system is believed by Kowalevski to arise from a portion of this plate. In the molluscs the pedal nerve cords arise posterior to the mouth and if the

1. Comparative Embryology Vol. II, page.

groove in *C. Polii* does form anterior to the blastopore and the resulting plate occupies this position it is difficult to understand by what method the nerves form from the plate and occupy a posterior position. It scarcely seems probable that such profound changes occur in *C. Polii* and not in *Ishnochiton* nor in any of the annelids and molluscs thus far described. I would suggest that owing to the difficulties in the way of the observation of the embryos of *Chiton Polii*, Kowalevski has wrongly oriented the earlier stages. Accordingly the so-called anterior groove and later tube are probably structures posterior to the blastopore. In some cases in *Ishnochiton* development the posterior third quartette stomatoblasts do meet on the median ventral line as in fig. but the tube in such cases is very short and soon disappears, not by a fusion of its walls, but by a species of evagination in which the cells forming the bottom of the groove come out level with the ectodermal cells of the surface of the body. So in *Ishnochiton* the walls of the tube never fuse and the mouth never completely closes, and if these processes do occur in *Chiton Polii* they are inexplicable

in the light of the development of any nearly related groups.

Gasteropods. The accurate study of the formation of the stomodaeum has not been made in any mollusc hitherto, and consequently comparisons are not possible beyond the earlier stages. Diagram shows the form of the blastopore in *Crepidula* at the latest stage in which the position of the second and third quartettes have been determined. The stages leading up to this point have been almost identical with the processes in *Ishnochiton* (cf. page) and in view of the fact that other later resemblances occur in this quartette it does not seem to be claiming too much to say that very probably the second and third quartette give rise to the stomodaeum as in *Ishnochiton*.

Annelids. In the light of many other resemblances between the embryos of *Nereis* and *Ishnochiton* it is impossible to believe that the similarity of the vegetative poles in these two forms is accidental (cf. diagrams). The blastopore is quadrangular in both and the secondary stomatoblasts lie in the angles; the third quartette con-

poses its sides; and within the space thus enclosed are the macromeres. On the posterior side of the Nereis embryo modifications have arisen but it is still easily possible to compare these with similarly located structures in chiton. These secondary changes, consisting primarily in the excessive development of the posterior second quartette cells (first somatoblast) are responsible for the changed position of the cells, the tertiary stomatoblasts becoming widely separated, yet they remain in the same relative position as in Ishnochiton.

As the ectodermal cells bounding the blastopore converge toward a central point as they do in Ishnochiton, the tertiary stomatoblasts, which have undergone several divisions, lie either wedged in between the secondary or pushed before these become located upon the macromeres within the blastopore. Their subsequent fate has not been determined further than that they do not contribute to the formation of the mesoblast. However in proving this point Wilson figures a number of stages which show in the clearest manner that several of the tertiary cells become enclosed within the circle of sec-

ondary stomatoblasts, therefore within the limits of the blastopore, and that very soon after this stage a small opening, the mouth arises within the circle and some of the third quartette cells form the roof of a shallow arch-enteron.

In comparing these tertiary cells with the third quartette stomatoblasts of *Ishnochiton* it is seen that they arise and are located similarly and considering their relatively small size they have very much the same development, and it appears very probable that these cells as the secondary are known to do enter permanently into the stomodaeum.

Now that it has been seen that the formation of the stomodaeum in annelids shows many points of resemblance to that of *Ishnochiton*, and that there are strong reasons for believing that the second and third quartettes enter into its formation it is rendered more probable that in its later development *Crepidula* follows along the same line. Indeed I believe it will be found the rule among gastropods and annelids that the stomodaeum forms from both second and third quartette cells.

The Foot.

Shortly after the blastopore comes to be situated immediately posterior to the velum the foot, more or less quadrangular in outline, arises as a median undivided protuberance on the ventral surface (fig.). Faintly demarcated at first it gradually grows in prominence by a deepening of the surrounding groove and as this process is taking place contractile movements commence to manifest themselves along its anterior border. As they extend themselves to all parts of the organ and about the close of the free swimming period the foot reaches its maximum of contractility, changing its shape with a rapidity that is never again met with in its history.

It is composed of high columnar cells, all of about the same size, which become clothed with fine cilia before the embryo leaves the chorion. The exact origin of the cells is open to some doubt, yet the diagram is not far astray probably. Certain it is that the greater portion of the foot is derived from the first somatoblast and the two bordering groups of third quartette cells which have formed the ventral side. The doubt arises as to the part the anterior third quartette products play in its formation. But since its anterior border is com-

paritively wide and in contact for some distance with the velum it appears probable that its antero-lateral borders arise in the territory of the anterior third quartettes.

Living embryos, especially during the free swimming stage, show a depression near the anterior border and in preserved specimens this may appear until the shell is nearly formed. A projection thus exists anterior to the depression and posterior to the mouth such as is shown in figs. . Sections show that along almost the entire edge of the projection the opening of the "foot-gland" (Kowalevski) is situated. This is scarcely to be spoken of as a true duct, but simply a series of more or less clearly defined intercellular channels which do not appear sharply defined except in sections.

Subjected as the young are to the violence of the waves I am of the opinion that Kowalevski is correct in speaking of this problematical organ as a foot gland, and that its secretion enables the organism to regain its foothold. Young chiton from ten to twenty days of age are with much difficulty removed with a camel's hair brush from the rocks on which they rest, and with later stages it is necessary to immerse them in a killing fluid before they can be dislodged.

The Mesoblast.

The mesoblast arises from the posterior macromere in the 72 cell embryo. Its first division occurs in the cell stage, and is generally bilateral although in many cases the spindle indicates a slightly isotropic cleavage (fig.). The next division, affecting both cells simultaneously, occurs normally in the stage shown in fig. . The spindles when fully formed are directed inward toward the centre of the embryo and the resulting blastomeres are situated in contact with each other in the median line, on the dorsal side of the parent cells abutting against the macromere B (diagram). This cleavage is perfectly bilateral as is manifested not only in the position of the spindles but in the movements of the centrosomes previous to division. In fig. the spindles are not perfectly formed and serve to show in what direction the centrosomes moved.

At a stage represented in fig. the next division of the parent cells occurs. In the direction of the spindles and the size of the resulting cells this cleavage is a repetition of the preceding. I have been unable to de-

termine from surface views the position of the daughter cells but a wax reconstruction shows them to be located between the parent and the first formed cells. Thus two mesoblastic bands exist but they are in close contact with one another and consequently do not diverge, as is the case in some annelids and molluscs.

Beyond this point I have been unable to follow the development of this germ layer. Invagination has proceeded to such an extent that the cells are no longer visible from the exterior and the lack of clearly defined cell boundaries and nuclei render it impossible to trace the development from surface preparations.

Later stages show the mesoblast to form a continuous sheet of cells extending from the posterior border of the blastopore along the dorsal surface of the embryo. This gradually extends laterally and anteriorly. Several isolated cells occur at various points but whether they have been derived from the primary mesoblast or as larval mesoblast I cannot state, though I hope a more extended study of sections may yield a definite answer.

The Entoblast.-The Fourth Quartette.

The history of 4 D (mesoblast) has already been considered and it now remains to trace briefly the history of the remaining cells of this generation, all of which enter into the formation of the mesenteron. The origin of these cells and their behavior in the process of invagination has already been considered up to the time of their division, and possibly for a greater period, in contact with the secondary stomatoblasts.

The only division of this quartette which I have observed occurs simultaneously in all the cells (fig.) The spindle in 4 B is perfectly meridional with its inner end almost in line with the animal pole. In 4 A and 4 C on the other hand the spindles are nearly horizontal, and the anterior ends of the spindles are directed to the left. The position taken by the daughter cells has not been observed.

The Fifth Quartette.

Invagination has advanced to a considerable degree and the macromeres have pressed into the segmentation cav-

ity and are in contact with the ectoderm of the velar field before the spindles arise which produce the fifth quartette. The macromere D shows no sign of division but the three remaining cells divide simultaneously, the spindles being all directed toward a common point located about the centre of the velar field. A considerable shifting occurs among the macromeres and the newly formed fifth quartette which renders it impossible to accurately trace their history into later stages.

The Free-swimming Larva.

It has invariably happened that no matter how carefully taken care of, the eggs of *Ishnochiton* will not segment normally after being kept two or at least three days in the aquarium. It is therefore not possible to take an egg through from the early stages until the embryo leaves the egg membrane, if it be kept in the laboratory, but if the egg strings soon after they are laid are carefully fastened among the corallines in some comparatively quiet tide pool they will then develop normally.

The length of time from egg laying until the embryo becomes free is seven days, and if one brings in the jel-

ly masses on the seventh morning after their deposition the larvae will be seen leaving the egg envelopes in gradually increasing numbers until about noon, and in diminishing numbers until evening.

In the period comprised between the commencement and completion of the shifting of the blastopore the embryo has a somewhat elongated form but in later stages an increase in size takes place, whereby the chorion becomes almost completely filled and the larva becomes more spherical as a result. Immediately preceding the free swimming stage spasmodic contractions and elongations of the trunk region are noticed and it is possible that in this way the membranes become ruptured and the larvae escape. These latter are heliotropic to the extent that if the water be agitated until they are distributed equally through the jar they will collect at the lighted side within fifteen minutes.

As has been seen strong cilia develop within twenty four hours after the first cleavage and in a 36 hour embryo the cilia beat with a rapidity of about 100 times per minute. This continues for five days and when the

larvae are liberated it would be supposed that with such a powerful locomotor apparatus they were destined to a free swimming existence of considerable duration. But in an apparently nearly normal environment this is not so. In almost every case when small lots were separated from the main company, they were found to settle within a period lasting from fifteen minutes to three hours. That this is apparently a correct and normal characteristic is shown by the movements in another chiton. It probably belongs to the genus *Middendorfia* and as in the case of *C. Polii* carries its eggs in the mantle furrow. In several instances I have found scores of small chiton within a very small distance of the parent, showing that the free swimming stage could have been only of the briefest duration. Certainly the chances of destruction are greatly lessened where the free swimming stage is short and it seems quite probable that a suppression of this period of the chiton's existence is a provision to insure greater safety and consequently to bring a greater number of individuals to maturity.

The movements of the larvae are of considerable

rapidity, the usual rate as based on observations of nineteen specimens being 8-10 c.m. per minute. In addition to this progressive motion is one from right to left, the animal thus moving in a loose spiral.

The flagella at the anterior end of the body at the centre of the velar area are generally two in number though as many as four occur. One is generally longer than the other, and after treatment with fixing agents they are seen to be compound, being composed of a number of lesser flagella. As the animal swims through the water these are whipped about in various directions, much like the great antennae of some of the crustacea, the base being held comparatively rigid in each case. At least one of its functions appears to be that of a sense organ. I have frequently noticed that the larva turned aside from a foreign object as soon as it was touched by the flagella and in such cases the body itself never came in contact with the obstacle.

It is with comparative difficulty that the conditions are made favorable for a further development of the larvae. If they are placed on ulva the greatest care

must be taken to change it frequently and even then the larvae soon commence to develop abnormally. I have found that somewhat water-worn shells of *Lytilus* afford the best material for the young to rest upon. In this case however the shells must be replaced at least every two days by others: the debris that collect about the young must be carefully removed with a camel's hair brush: and also in case the sea water is at all filled with sediment organic or inorganic it is best to pass it through a filter before allowing it to run into the aquarium.

At first the larvae half crawl and half drive themselves along by means of their cilia and frequently leaving the object upon which they rest they swim about again. In such cases it appears that the resting place was unfavorable; at all events it is readily seen that some places on the shell or ulva are closely packed with settled larvae while other equally accessible portions are nearly or quite bare.

For some time after the larvae settle they remain quite active, contrasting strongly with the slow steady moving embryos a day or two older. If disturbed or irri-

tated they will sometimes bend the head vesicle either to the right or left or dorsally almost at right angles to the trunk. This occurs until the shell has commenced to form but after the portions of the valve have fused into continuous plates such movements entirely cease.

Metamorphosis.

As may be readily seen the metamorphosis of a free swimming larva in assuming the adult characters is relatively slight, and concerns almost altogether the head vesicle. At first this is almost hemispherical, but later it becomes pear-shaped or conical with the tuft of flagella at its narrower end.

The shell occupies a portion of its upper surface and in its subsequent changes this region undergoes little change of shape while the remaining portions of the head vesicle undergo modifications which result in the formation of the anterior portion of the mantle and the proboscis.

A day after the larva has left the egg membrane the anterior hemisphere shows no very decided permanent change of form although it is constantly changing its shape and

exhibits a slight tendency to become more blunt. This latter feature is probably aided by the first valve increasing in extent. During the next day the shell advances still farther the velum is cast off and the lower surface of the head vesicle becomes flatter giving the embryo in side view an appearance shown in fig. . About this time the anterior prolongations of the mantle furrow appear in the head vesicle below the shell (fig.). It is shallow at first but gradually deepens bringing into prominence the area which becomes the proboscis. At this stage the mouth lies posterior to this area but gradually pushing forward it comes to lie in the centre of the developed snout. At this time the embryo appears as in figure . The rounded convex appearance of the proboscis rapidly disappears and the condition shown in figure is reached.

Thus the head vesicle becomes transformed into part of the first valve of the shell, the mantle and mantle furrow of the same region, and the proboscis, and as will be remembered these are thus derived from the first quartette of ectomeres.

In the trunk region few changes take place; the

mantle furrow becomes deeper and the foot more differentiated. When the anus breaks through I cannot say definitely for *Ishnochiton*, but I know that in the 15 day embryo this process has not occurred. In another species (*midcendorfia*) however this process takes place at about the time corresponding to the 15 day stage of *Ishnochiton*.

As to the gills I may say that they arise as minute papillae in the mantle furrow, and at a relatively late period several days beyond the formation of the proclodaeum.

Cell homologies among Annelids and Molluscs.

Of late years a growing tendency is manifesting itself to look upon the early cleavage stages as something more than a mere manifestation of simple mechanical forces. Rather are the blastomeres the expression of the action of intrinsic forces which control the development from the earliest stages on to the end. Mechanical forces and conditions such as gravity, surface tension, cohesion and pressure undoubtedly are operative, but they are not believed to be the controlling, coordinating agents in development. The early cleavages accordingly are as important as those occurring in later life, and may even be considered more so (cf. Watasé). Also the long

continued resemblances which exist in the development of flatworms, annelids, and molluscs, from the earliest segmentation of the egg, are as fundamental and deep seated as are the homologies existing in the adults.

The list of these resemblances in annelids and molluscs is constantly increasing. In all forms accurately studied the first three quartettes of cells constitute the sum total of the ectoblast; the mesoblast arises at the fourth division of the posterior macromere; the remaining members of this quartette and the macromeres become entoblast; while the divisions and positions of the cells up as far as the 26 cell stage are identical. Beyond this point Wilson ('92) believed a divergence between the two classes ensued, and that the development proceeded upon two entirely different lines. However subsequent observations have not confirmed this belief, but have rather served to show that the supposed differences were superficial or non-existent and therefore the points of resemblances became more numerous and extended throughout a longer period of development.

The above characters have been shown by Billie ('95)

to exist in the lamellibranchs, and also in both classes there is an essential similarity between the development of the first somatoblast. In annelids this structure develops to a greater extent than in *Unio* but as will be seen the two have many points in common.

Mead ('97) , in a comprehensive study of the development of five annelids, recognized the above resemblance and presented other important points wherein particularly the annelids agree. The rosette series was shown to have the same origin and position in both annelids and molluscs, and it was considered in the highest degree probable that in both it gives rise to the apical sense organ, but whether its final development resulted in the formation of similar organs remained an open question. The primary or secondary trochoblasts were shown to have identically the same fate in at least two annelids: the head kidneys in *Amphitrite* develop from the same cells as in *Nereis*: the entoderm arises from the same cells in all cases.

In a paper appearing shortly after the foregoing, Conklin ('97) confirmed the correctness of the above list

previous to Mead's work and added several other points to the list of resemblances, some of which have been mentioned by Mead. In addition it was shown that the primary trochoblasts of molluscs are, at least in part, precisely similar in origin and destiny to those of annelids; that the cerebral ganglia probably arises from the same group of cells in both; that the axial relations of all the blastomeres with the possible exception of the macromeres are the same in each; and finally that the larval mesoblast of *Crepidula* arises from the same group of ectoderm cells as in *Unio*.

The above list consists of no less than twelve points wherein there is an essential similarity in the development of annelids and molluscs, and the development of *Ishnochiton* with its more direct and less involved development serves in the most emphatic manner to prove the correctness of these observations..

In addition to the above resemblances, I would once more emphasize the wonderful homology which exists between the annelid and *Ishnochiton* prototroch, in which twenty-two of the twenty-five cells in the former have exactly the same origin, direction of cleavages and destiny as in

Ismochiton, and the remainder of the first quartette forming the head vesicle, with its rosette series and molluscan cross cells or intermediate single cells, has in all probability the same fate in both.

The second quartette has been shown to have exactly the same origin and relations in both classes and several of the early divisions are similar. In some forms precocious segregation has produced excessive modification in 2d- so great that it appears at first sight almost impossible to reconcile the differences that have arisen; but a more careful study shows conclusively that they are but variations of a common radial type and from this standpoint it is possible to trace and understand a remarkable series of resemblances that appear likewise inexplicable.

In the invagination, modifications have likewise arisen. Accumulation of yolk and precocious segregation have both been instrumental in producing many changes, but whether the gastrula be produced by epibole or embole the closest resemblances appear. In all cases the macromeres and three of the fourth quartette products produce the

mesenteron and the relation of these cells and their behavior during gastrulation are very similar. Also the mesoblast in its position and development exhibits fundamental resemblances throughout. The blastopore is usually quadrangular with second quartette cells in the angles and third quartette forming the sides, and during gastrulation some of these second quartette products become invaginated in *Nereis* and *Ishnochiton* and enter into the formation of the stomodaeum; the same thing is true to a greater extent of the third quartette in the latter form, and probably also in *Nereis*. These are the only animals in which the formation of the stomodaeum has been accurately followed and it may be a little early to formulate any sweeping generalization, yet from the behavior of the same cells in other forms as far as these have been followed the resemblance is so close that it leads irresistably to the belief that in several other forms the second and third quartette both contribute to the formation of the stomodaeum.

As a result of shifting the mouth comes to lie immediately posterior to the prototroch, this latter organ

separating the first from the second and third quartette of ectomeres.

In the velar field there is reason to believe that a portion of the rosette series develop into the cerebral ganglia, and finally the interesting fact appears that the annelid prostomium is the homologue of that portion of the animal including the proocephis, the anterior portions of the first valve, and the mantle furrow anterior to the eye. The remaining portions, the trunk and alimentary canal, have been shown to conform to the same fundamental type although secondary modifications have arisen which tend to obscure the fact.

Thus it is seen that not only in the origin and position of the various quartettes do resemblances appear but that the early cleavages of these are in many cases cell for cell the same. In the later stages close cell homologies cease, but the realition of the cell groups and their development in giving rise to larval or adult structures follow along much the same path. After passing these facts in review and considering the various structures in detail, and the modifications which they under-

so one fact presents itself with the greatest clearness—that between *Ishnochiton* and the annelids the resemblances are more fundamental and closer than are the differences.

Ancestral Form.

It is a fact of common observation that in the development of annelids, flatworms and molluscs the embryo in its earlier stages exhibits a radial symmetry. This in some forms is somewhat modified owing to a precocious segregation of the material that will subsequently enter into the first and second somatoblasts, but a four cell stage exists and from these blastomeres three quartettes of ectomeres arise. In such cases however the radial symmetry, imperfect from the first, is soon superseded by the permanent bilateral form. In other cases where the precocious segregation is not manifested in the early stages a complete radial symmetry exists which ultimately becomes modified into the bilateral type, but at a much later period than in the first case.

Ishnochiton presents a remarkable example of this latter type: its quadriradial symmetry is in the highest degree apparent. Three quartettes of ectomeres arise in

as many cleavages of the macromeres, and the structures to which they give rise-the annelid and gastropod crosses,

the three species of trochoblasts, the groups of second and third quartette products- all, for a considerable period, conform to the radial type. Also the ectomeres and macromeres, scarcely less perfect in this regard, aid in the formation of a beautifully symmetrical embryo in which all quadrants are essentially identical.

The first changes which creep in relative to the shifting of the embryonic axis are due wholly to accelerated cleavages. We have already seen that this early effects deep seated modifications in the first somatoblast, but in the other ectomeres the change is much more gradual. For example, the acceleration in the divisions of the posterior third quartette cells is small at first, and it is not until there are over two hundred cells in the embryo the difference between the groups becomes strikingly apparent. And as regards the cells in the anterior hemisphere, a glance at figure in the 2 cell stage will show that the radial symmetry is but slowly giving way to the bilateral.

In Nereis and Amphitrite, on the other hand, the bilateral condition has become reflected backward onto the early stages to such an extent that it manifests itself at the first division-and later in strictly bilateral cleavages various groups of cells. But in the case of Lepidodotus and Ishnochiton this is by no means so apparent, and it is only by the aid of certain landmarks that one is able to orient the embryo up to the 64 cell stage, and in Ishnochiton as we have seen the radial condition becomes but slowly modified into the bilateral.

The question now arises ,what does this early radial condition represent? A group of cells of no morphological value or of the utmost fundamental importance in the formation of the embryo? I believe many facts in the development of Ishnochiton and other forms lead to the belief that the truth lies on the side of the latter proposition. That the original ancestral form of the trochophore was a quadriaxially symmetrical organism whose principal axis corresponds to the axis of the gastrula, and that the shifting of this axis is secondary.

The original ancestor therefore may be compared to

a highly modified gastrula, or gastreaa, so far at least as these three groups of animals are concerned, in which the mouth was situated at the vegetal pole; and an ectodermic pharyngeal portion of the alimentary canal (stomodaeum) existed in connection with the entodermic mesenteron. At the opposite end an apical sense organ probably existed which was situated at the intersection of the quadriradial nervous system occupying the upper hemisphere. Encircling the entire animal was an equatorial band of cilia. All of these features appear during development in varying degrees of perfection, and possibly others may have existed originally but the embryology gives no certain answer regarding their character. Subsequently in the phylogeny the mouth shifted its position, due it is believed to the advantage of coming into close proximity of the prototroch which served as a food¹ procuring apparatus.

" Many developing embryos during the first stages of this shifting show a narrow slit extending posteriorly from the blastopore which is looked upon by some investigators

1. Cf. Entwick. der Wirbellosen Thiere, page 408, Schacht u. Heider.

as indicating an ancestral character, but this is by no means apparent. In several cases this feature scarcely exists at all and where it is strongly developed it appears to be secondary.¹ And neither does it appear correlated with the formation of the anus, at least not in chiton, where this latter organ does not form until fully fifteen days have elapsed. Ultimately the mouth becomes situated immediately posterior to the prototroch, and thereby the former radial symmetry became in large part modified in later stages, although traces of it still exist, as development of modern forms clearly shows.

Returning to the phenomena of early development it may be said that since they undergo a similar shifting of axes in their development it is reasonable to suppose that polyclades, annelids, and molluscs diverged from the ancestral trochophore after it had become bilateral. Therefore, theoretically, all the embryos in their on-

1. Child ('97) has recently shown that the lips of the blastopore condescend owing to the growth of the somatic plate.

togeny should pass along the same developmental path up to the assumption of the bilateral form. That this does not actually happen is owing probably to secondary factors, such perhaps as the reflection of the bilateral form upon the earlier stages (precocious segregation) and accumulation of yolk etc., which tend constantly to modify this ancestral condition. Hence when bilateral cleavages appear in development previous to the shifting of the blastopore they may be considered secondary, and in many cases they show that they are such, exhibiting unmistakable modifications from a radial type. And as has been maintained above, it does not necessarily follow that these secondary bilateral cleavages everywhere follow the same path, for even though they have been moulded upon similar radial forms they tend to depart from a common type. And in the comparison of the development of an egg with an abundance of yolk where gastrulation is epibolic, with one with smaller amount of yolk and an embolic gastrulation, yet in spite of modifications produced by precocious segregation and the effects of food yolk remarkably close resemblances occur between the embryos

of annelids molluscs and polychaetes and assuredly point to a close relationship.

Forms of Cleavage.

Growing out of the above considerations some points arise relating to the significance of radial and bilateral types of cleavage. As I have said above I believe that the early radial condition of the embryo is of fundamental significance; that it represents an early primitive condition phylogenetically when the ancestor of the trochophore was a radially symmetrical organism. The trochophore as we recognize it today has been modified from this condition owing to the formation of a ventral surface and correlated shifting of the mouth, and one of its most distinctive features is its bilateral form.

In development the organism passes from a radial to a bilateral condition, a process which in *Ishnochiton* is of greater duration than in some annelids (e.g. *Nereis*, *Amphitrite*, *Arenicola*) or in snails among the lamellibranchs. That is to say, *Ishnochiton* longer retains its primitive characters than these forms in which the radial structure

8.
Liberals
as primitive
a radical effect

is soon replaced by the secondary bilateral. And in those forms where precocious segregation is most pronounced the radial symmetry is as a rule proportionately decreased, from which it follows that the reflection of larval stages upon the early stages does not produce radial symmetry but tends to destroy it. There is a warfare so to speak, a contest between the radial and bilateral condition wherein the latter appears to be slowly replacing the former, and it has even gone so far as to produce marked effects in some cases at the first cleavage, and theoretically if not actually (cephalopods) bilateral cleavages may arise from the beginning and no trace of the radial exist.

Where this bilateral condition appears in the embryo it is in connection with bilateral cleavages and I heartily agree with Conklin when he says "the bilaterality of cleavage is only an early appearance of the final bilaterality with which it is directly continuous". But I believe that when he says "in several cases these radial structures" (speaking of the radial symmetry of the quartettes, trochoblasts, apical rosette, molluscan cross

etc.) seem to belong to the same category as the radial structures of the trochophore larva, and I believe that they are to be explained as a foreshadowing of larval characters, just as bilateral cleavages are usually attributed to a precocious development of adult characters; it holds good not for both radial and bilateral characters but simply for the bilateral. Rather may it be said that bilateral prelarval stages of annelids and molluscs may be held to be due to the similarity of their larvae, but considering the radial form of the embryo I believe that the problem has been attacked from the wrong side. I believe rather that the radial symmetry found in the trochophore has persisted in spite of the bilateral condition of the larva, and that it belongs to a period in the phylogenetic development of the embryo which preceded the bilateral. Therefore just as bilateral cleavages are due to a bilateral condition, so I believe radial cleavages are due to radial symmetry.

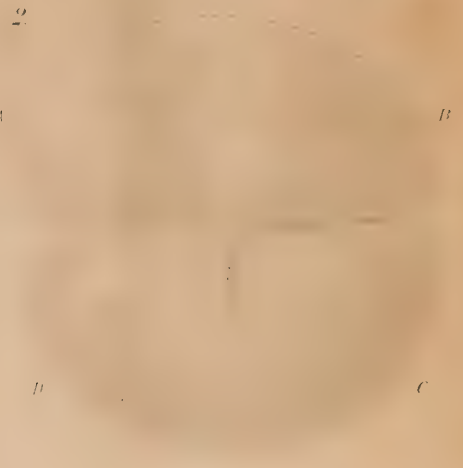
While the formation of a ventral surface gave rise to a bilateral form it does not follow that bilateral cleavages arose at that time. On the other hand it ap-

pears that at least in the ectoderm and entoderm these have arisen at a later period. And neither does it follow that in the early history of the radial ancestor the cleavages were radial. Rather it appears to me that, as in the case of bilateral cleavages, the radial divisions gave a greater directness to the development, and arose from a saving of energy which takes place, as Conklin holds, whenever precocious segregation is present.

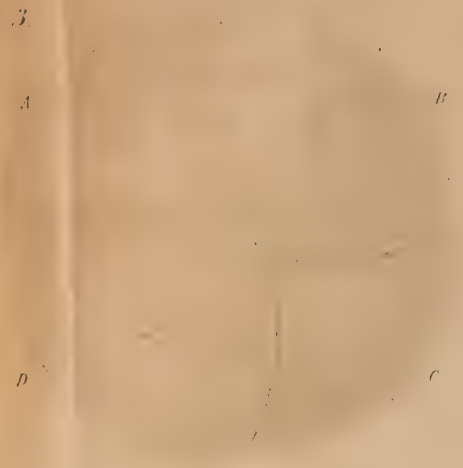
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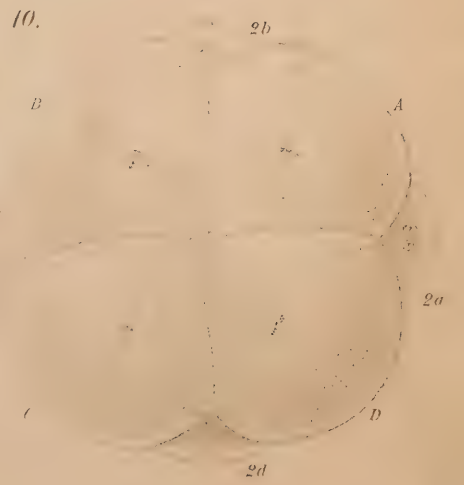
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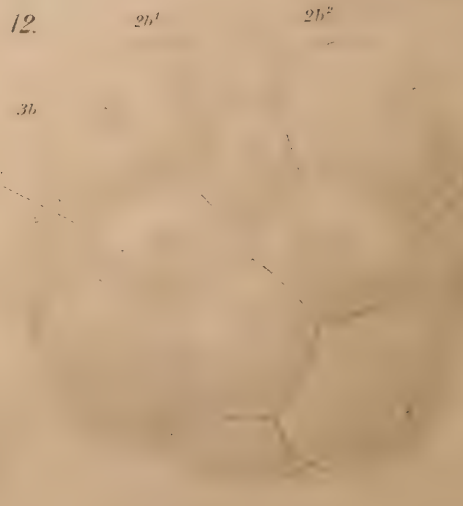
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Gustav Fischer

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Fig. 1

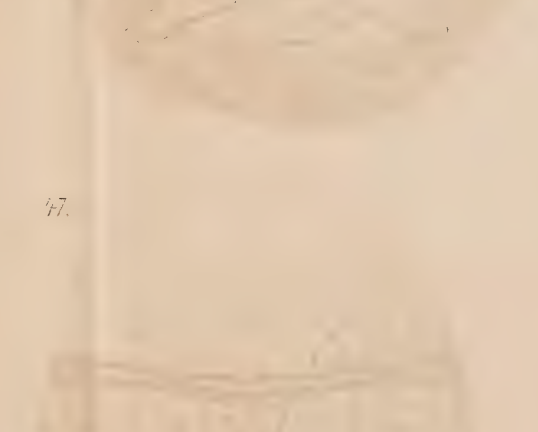
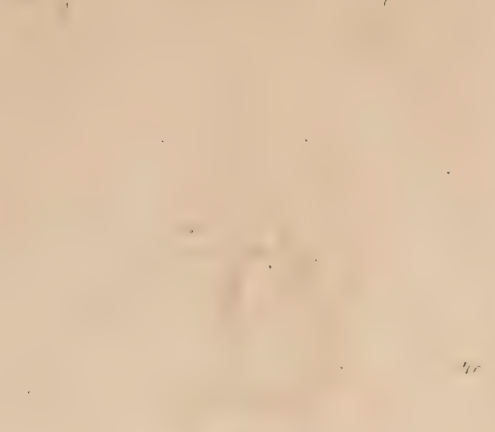
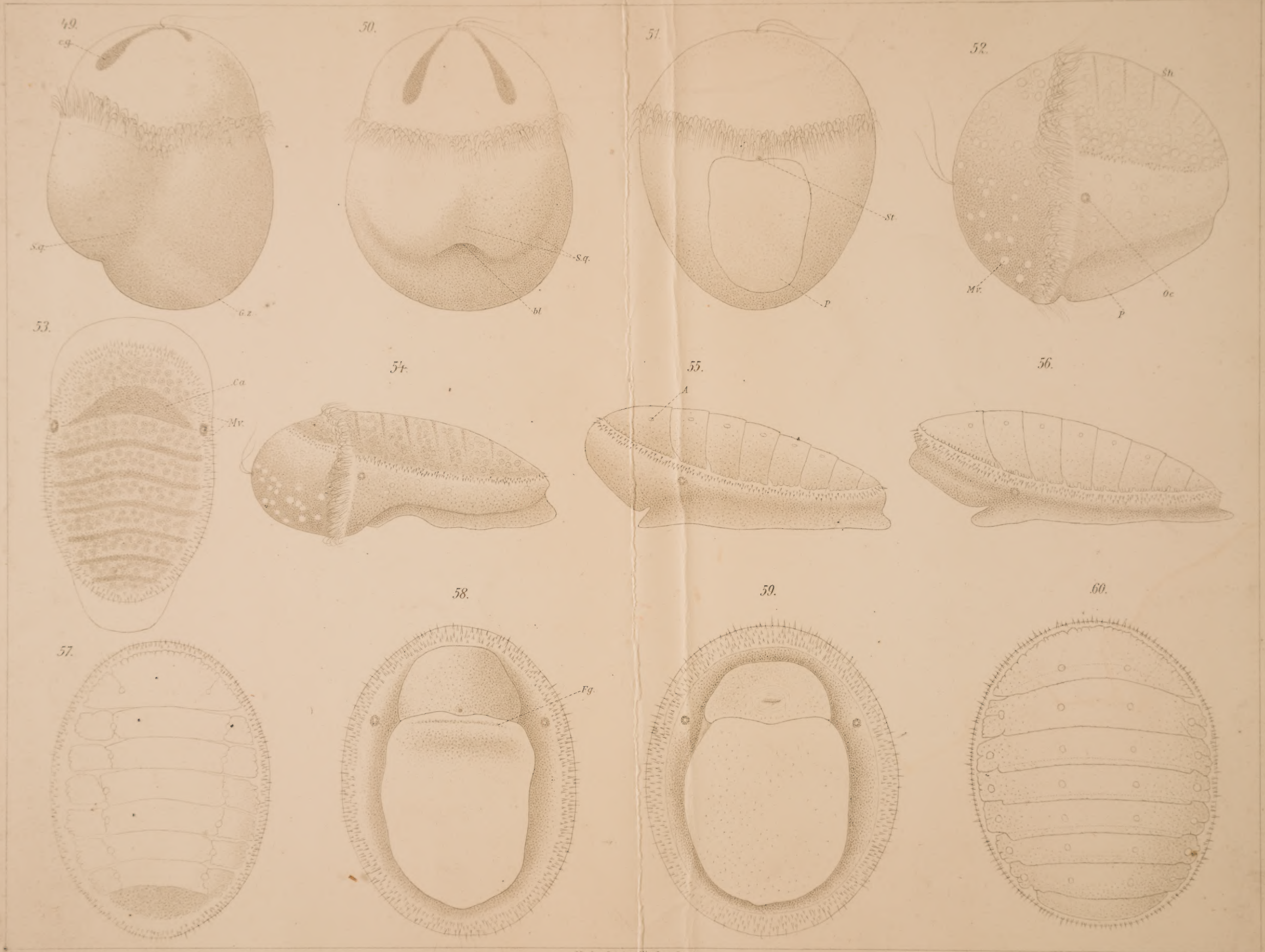


Fig. 2

Fig. 3





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